THE SUPERFICIAL GLANDS OF THE ŒSOPHAGUS.

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PLATE XXI.

The glands of the œsophagus are of two varieties. Of these the submucous glands have long been recognized. F. A. Schmidt[1] in 1805 described them and there have been many descriptions since then. They are typical mucous glands situated in the submucous coat of the œsophagus (Plate XXI, Fig. 2). Their ducts pierce the muscularis and frequently show cystic dilatations before emptying by their comparatively narrow mouths. The ducts are lined for some distance from their orifices by a stratified epithelium of cuboidal cells. They frequently pass through lymphatic nodules or lie close to them. The secreting cells and the contents of the ducts stain deeply with stains for mucin.

The other variety of glands is totally distinct from these submucous glands, and as they are best characterized by their situation superficial to the muscularis mucosæ, I shall speak of them as the superficial glands of the œsophagus (glandulæ œsophageæ superficiales). They occur mainly in two localities in the œsophagus: (1) in areas in its upper portion, and (2) at the transition of stomach and œsophagus. These latter, the so-called "Cardiadrüsen," extend but a short way toward the stomach, being soon replaced by the typical fundus glands. Toward the œsophagus they may extend a short distance beneath the stratified epithelium or occur in little groups just above the gastro-œsophageal junction. They have been grouped by some with the gastric glands. The areas of superficial glands which

[1] De mammalium œsophago atque ventriculo. Inaug.-Diss., Halle, 1805.

are found in the upper œsophagus will be considered especially in this paper.[2]

Rüdinger[3] described these glands in 1879 as tubulo-acinous glands of the œsophagus lying above the muscularis mucosæ. According to his description they are situated in the lateral wall of the upper part of the œsophagus but are not bilateral. He describes them as consisting of three portions: (1) narrow peripheral tubules lined by pyramidal cells; (2) large cavities into which the tubules empty; (3) the ducts to these cavities which lead to the surface. The cavities and ducts are lined by high cylindrical cells. W. Krause[4] probably had Rüdinger's work in mind when, in 1879, he classified the œsophageal glands as (1) the common, isolated glands in the submucosa, (2) the smaller glands in the mucous membrane of the lower œsophagus, and (3) a tubular variety of glands at the upper end of the œsophagus. In 1887, Lauteschläger,[5] after examination of several œsophagi, was unable to confirm Rüdinger's observations.

With these exceptions, Rüdinger's work had passed unnoticed and these glands had been overlooked or misinterpreted, until J. Schaffer,[6] in 1897, again called attention to them. He had his attention directed to an area 6½ mm. by 4 mm. in the œsophagus of a girl eleven years of age. This proved to be made up of glands superficial to the muscularis mucosæ. He found similar though smaller areas in other œsophagi, but he was unable to find the glands in one œsophagus, from the upper part of which serial sections were made of a piece 1½ centimetres long. He found the glands to be bilateral and located between the levels of the cricoid and the fifth tracheal cartilage. He describes the glands as consisting of a number of twisted and branched tubules of varying diameters

[2] Schaffer calls these glands "die obere Cardiadrüsen," but as the glands in the fundus of the stomach have been designated by some English writers as "the cardiac glands," I have, in order to avoid the suggestion of identifying these œsophageal glands in structure with the latter, preferred the term "superficial glands of the œsophagus" to Schaffer's designation.

[3] Beiträge zur Morphologie des Gaumensegels und des Verdauungsgsapparates, pp. 27–31. Stuttgart, 1879.

[4] Handbuch der menschlichen Anatomie, ii, p. 445. Hannover, 1879.

[5] Beiträge zur Kenntniss der Halseingeweide des Menschen. Inaug.-Diss., Würzburg, 1887.

[6] Ueber die Drüsen der menschlichen Speiseröhre. *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1897, cvi, p. 175. Beiträge zur Histologie menschlicher Organe. Ibid., cvi, p. 403. Epithel und Drüsen der Speiseröhre. *Wien. klin. Wochenschr.*, 1898, xi, p. 533.

lined by low cylindrical or cuboidal cells. Among these cylindrical cells he found parietal cells in varying numbers in each of his specimens. These tubules empty either directly or by means of wide spaces into the duct. The duct may be dilated, but its opening is always narrow and it always comes to the surface at the top of a papilla. When several such ducts have a common place of emptying, the stratified epithelium is pushed aside and the area is covered by columnar epithelium. Schaffer showed that these glands are morphologically identical with the glands found at the transition of the œsophagus and stomach (Cardiadrüsen).

On the same date that Schaffer presented his first article, Eberth[7] described an area in the œsophagus which he interpreted as misplaced gastric epithelium. It presented a round, reddish and well circumscribed surface about the size of a five-Pfennig piece at about the middle of the œsophagus. On microscopic examination it was seen that the stratified epithelium ceased abruptly, being replaced by a great number of tubular "mucous" glands analogous to those in the stomach. Eberth did not find similar areas in other œsophagi. Although this area was at a lower level than is common for the superficial glands there seems no doubt but that it was such a glandular area.

Oppel,[8] in a review of Schaffer's work, speaks of the interesting comparison between the superficial glands of the upper œsophagus and the glands at the transition of the œsophagus and stomach. He deems it important to have a confirmation of Schaffer's work, and especially of the occurrence of parietal cells in the œsophagus. Hildebrand[9] in 1898 reported a single case of the "occurrence of gastric glands in the œsophagus," which he regards as identical with those described by Schaffer and by Eberth. In his case there were paired areas of glands in the upper œsophagus and these contained parietal cells. In the last edition of Quain's Anatomy[10] it is stated that a few of the smallest of the œsophageal glands are situated in the substance of the mucous membrane. Such are undoubtedly superficial glands. Böhm and v. Davidoff[11] describe the œsophageal glands as emptying at the apices of the papillæ, which is true only for the superficial glands and not for the

[7] Verirrtes Magen-Epithel in der Speiseröhre. *Fortschr. d. Med.*, 1897, xv, p. 261.

[8] Lehrb. d. vergleichenden microscopischen Anatomie d. Wirbelthiere, ii, p. 153. Jena, 1897.

[9] Ueber das Vorkommen von Magendrüsen im Oesophagus. *Münch. med. Wochenschr.*, 1898, xlv, p. 1057.

[10] Quain's Anatomy, edited by E. A. Schäfer and G. D. Thane, Vol. iii, Pt. iv, p. 66 London, 1896.

[11] Lehrbuch der Histologie des Menschen, pp. 170–171, Wiesbaden, 1895.

more common submucous glands. Their representation of an œsophageal gland [12] also appears to me to be a superficial gland and not a submucous gland.

In 1899, without previous knowledge of Rüdinger's or Schaffer's publications, the attention of Dr. Ophüls, Professor of Pathology and Bacteriology at Cooper Medical College, San Francisco, was called to these superficial œsophageal glands, by finding two corresponding "oval defects" in the mucous membrane of the œsophagus of a patient who died of pneumonia. These defects were upon its lateral walls, were symmetrical, and were opposite the upper tracheal cartilages, 5½ cm. below the opening of the larynx (Plate XXI, Fig. 1). The long axis of each, measuring 2 cm., was parallel to the long axis of the œsophagus; the width of each was 0.5 cm. The surface was somewhat lower than that of the surrounding mucous membrane and was smooth, glistening and dark red in color, so that these areas looked not unlike ulcers, although on account of their symmetry and regularity of outline this idea was dismissed and a congenital misplacement was suspected. Sections showed these areas to consist of tubular glands superficial to the muscularis mucosæ.

In ten consecutive autopsies, similar but smaller glandular areas were recognized in five cases macroscopically, and these findings were confirmed in each instance by microscopic examination. The size of the areas in these five cases varied from 3 mm. to 8 mm. in the longest diameter. They were circular or more or less oval, and when oval the long axis was parallel to the long axis of the œsophagus. The location in each case fell within the limits as given by Schaffer, namely, on the lateral wall of the œsophagus from the level of the cricoid to that of the fifth tracheal cartilage. They were noted to be bilateral in two cases. One specimen which was at first supposed to contain only a single area was demonstrated upon microscopic examination to present a corresponding smaller area on the other side. This shows how easily a few of these glands may be overlooked. The size of an area therefore may vary greatly: from one invisible to the

[12] Op. cit., Fig. 121.

unaided eye to such a large area as that in our first specimen. There may be only a few glands present, separated from one another by areas of connective tissue and smooth muscle, their ducts opening at separate places through the squamous epithelium. In such cases the squamous epithelium over the glands is of diminished thickness and the papillæ are either small or absent. Such an area appears in the gross as a small nodular bulging in the mucous membrane, usually somewhat larger than the elevations produced by the submucous glands. In other cases, the glands may be so closely packed as to simulate the gastric glands in appearance, although strands of connective tissue are still seen dividing the tubules into groups. In such

FIG. A.—Diagram of a simple form of superficial gland. Drawn from a reconstruction. x 70.

a case, the stratified squamous epithelium ends abruptly at the glandular areas, the Malpighian layer bending at almost a right angle and becoming continuous with the single layer of columnar cells covering the irregular surface between the mouths of the ducts (Plate XXI, Fig. 2). Such an area looks to the naked eye not unlike an ulcer with well defined edges. It presents a low, red, glistening surface that readily attracts notice on careful examination of the œsophagus.

The glands are of the branched tubular type. They are remarkable for their many windings as well as for their cyst-like spaces. It is rare to see a gland cut longitudinally for any distance on account of the irregular course which it pursues. The complexity of the glands and the number of branches varies greatly. Fig. A drawn from a reconstruction is a schematic representation of one of the simplest

of these glands if its branches were laid in one plane. It is seen to consist of a duct and several acini. The duct (Fig. B, d) is lined by

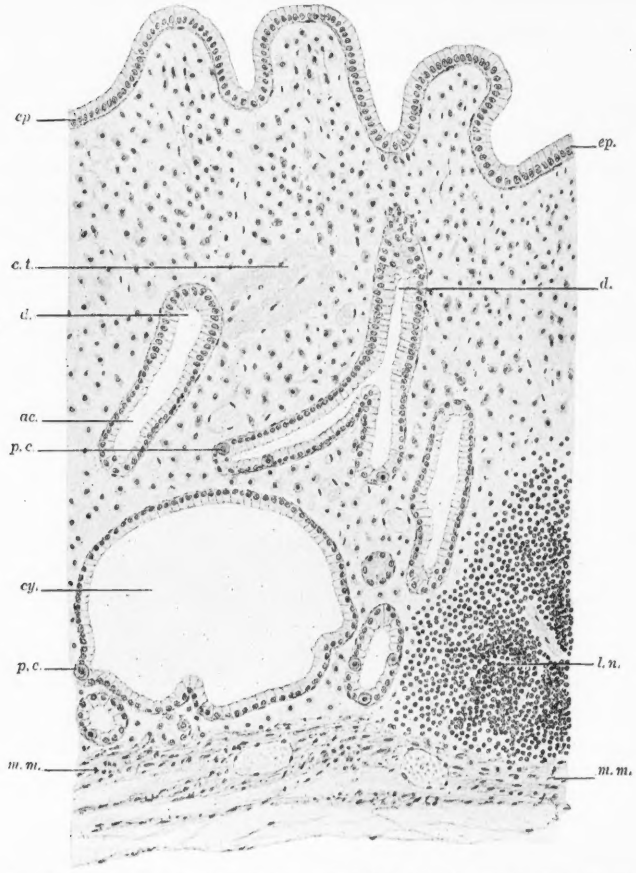

FIG. B.—Drawing of a section of the superficial gland area, x 130. *ep.* columnar epithelium of the surface, *d.* duct, *ac.* acinus, *cy.* cystic dilatation of an acinus, *p. c.* parietal cells, *c. t.* strands of dense connective tissue, *m. m.* muscularis mucosæ, *l. n.* lymphatic nodule.

high columnar cells two to four times as high as they are broad. The nuclei are oval with their long axes parallel to the long axes of the cells. They are situated all at about the same level, a short distance

from the base of the cell. A few ducts contain what appear to be goblet cells among the other cells. Where the gland begins to branch, the character of the epithelium changes. The cells become shorter and more conical. The nuclei become spherical or oval with the axis at right angles to the axis of the cell or even flattened transversely against the base of the cell. Their situation is uniformly nearer the base of the cell than is that of the nuclei of the duct cells (Fig. B, *ac.*, and Plate XXI, Fig. 3). The protoplasm of these acinous cells appears reticulated.

In the acini of many of the glands cells of another type are seen, identical with the parietal cells of the gastric tubules. These are round or oval cells which are usually readily distinguished on account of their property of staining deeply with eosin and picric acid. The nucleus is located at about the centre of the cell (Fig. B, *p. c.*, and Plate XXI, Fig. 3). These cells frequently possess two or three nuclei and occasionally a cell may have four or five. The cell body appears almost homogeneous in some specimens, but in others it is seen to be filled with small refractive granules densely packed together. Clear spaces in the protoplasm, apparently vacuoles, are of frequent occurrence in these cells. It will be seen that in all these particulars they agree in structure with the parietal cells of the stomach. Schaffer states that he found parietal cells in each of his first six specimens. I was not able to find them with such regularity and succeeded in demonstrating these cells in only three of six specimens. They certainly vary greatly in number in the different œsophagi, for in two cases they were present only in small numbers, while in our original specimen they were the prevailing cells in many tubules.

One of the distinguishing features of these glands is the presence of cystic cavities (Fig. B, *cy*). These were present in some of the glands of each specimen examined, but they showed great irregularity in size and shape. Their diameters varied up to 0.7 mm. The epithelium lining these cystic structures is usually like that lining the ducts of the glands. Other cysts, however, and especially those situated deeply in the mucosa, are lined by low columnar cells of the type found in the gland tubules, and, in addition, they may contain parietal cells (Fig. B, *cy.*; *p. c.*). Still other cysts have duct epithelium lining

their superficial portions and glandular epithelium lining their deeper portions. It would appear, therefore, that these cystic structures may be due to dilatations of different parts of the gland. That they are not present in every gland, as Rüdinger thought, is shown by their absence in the gland represented in Fig. A. The portion of the gland which is more commonly dilated is the duct and less frequently it is the secreting tubule, and this accounts for the variability in the cell-lining of the cysts. These dilatations are, as a rule, least frequent in the glands which are richest in parietal cells, and most frequent in glands containing no parietal cells. They are filled usually with a mucoid material containing desquamated cells and cellular debris. These cystic structures are comparable to the dilatations which occur in the ducts of the submucous glands. The function of the latter dilatations has been regarded as that of reservoirs which hold the secretion. As the food passes down the œsophagus the mucus is pressed out and so serves to lubricate the œsophagus at a time when it is most needed. The majority of the cystic structures in the superficial glands probably act in a similar manner. Others of these, however, can hardly serve this function. A careful study of serial sections shows that in many cases, especially in the deep-seated cysts, no demonstrable communication exists between the cystic cavity and the surface. Such are, in fact, small retention cysts. The spherical shape of the larger cysts also indicates that the contents are under some considerable tension. In one place a ruptured cyst was seen with extrusion of its contents.

As Rüdinger was the first to point out, the superficial glands lie above the muscularis mucosæ, therein differing essentially from the submucous glands of the œsophagus. The muscularis mucosæ, as it approaches the gland area, usually becomes diffuse and increases in thickness. Beneath the glands it forms a thin compact layer closely applied to their bases (Fig. B, *m. m.*, and Plate XXI, Fig. 2). Where the glands are some distance apart the muscularis mucosæ may accompany the connective tissue which runs in between them. In such a case the base of the gland is surrounded by muscular fibres and when these contract they probably compress the glands and so aid in expelling their contents.

Schaffer regards the absence of a marked mucin-staining reaction in the superficial glands as an important feature distinguishing them from the submucous glands. There is a mucoid material present in the ducts and cysts, but this does not give a decided mucin-staining reaction. However, in a small number of cells sufficient mucin is present to give a very definite reaction for mucin. These cells are mostly in the lower part of the glands and in my specimens were grouped in two or three little areas, so that probably only a few of the glands contained mucin-producing cells in their acini. Whether these glands are essentially different from those in which the cells gave no mucin-staining reaction was not determined. The mucin is situated in that portion of the cell which is toward the lumen of the gland. Certain cells in the ducts of the glands also take a slight mucin stain. Yet the mucin reaction of the superficial glands as a whole is very slight compared to that of the mucous glands of the submucosa. Even in hæmatoxylin specimens, not too much decolorized, the difference is plainly seen, the submucous glands being of a deep blue color. This is well shown in Plate XXI, Fig. 2.

Lymphatic nodules are present in considerable number in the superficial gland areas. They are located just above the muscularis mucosæ corresponding to their situation in the stomach and intestine (Fig. B, *l. n.*, and Plate XXI, Fig. 2).

The morphological significance of the superficial glands is not clear. Their most interesting feature is the possession of parietal cells, which have been regarded hitherto as the special property of gastric glands. It is not strange that when many parietal cells are present, the glands containing them should be mistaken for a misplacement of gastric glands. As an explanation of their origin in his specimen, Eberth suggests that the squamous epithelium in its growth downward from above circumscribed an island of columnar epithelium which in the growth of the œsophagus became widely separated from its fellow columnar epithelium in the stomach, but like it developed into characteristic gastric glandular tissue. E. Neumann[13] showed that there

[13] Die Metaplasie des fötalen Oesophagusepithel. *Fortschr. d. Med.*, 1897, xv, p. 366.

is not a migration of stratified epithelium from above downward but that it develops in situ. Schaffer points out that both the stomach and the œsophagus are derived from the foregut and that while in the œsophagus squamous epithelium is developed, in the stomach the epithelium remains columnar and develops the gastric glands. He considers that a portion of the œsophageal mucous membrane, in failing to change to squamous epithelium, may develop similarly to the gastric mucous membrane and so give rise to gastric glands. In support of his hypothesis Schaffer has described paired areas of simple columnar epithelium in the upper œsophagus of a three-months fœtus. He regards these as an early stage in the development of the superficial glands. Why should this lack of development occur with such frequency in the upper rather than in other portions of the œsophagus? It is well known that the developmental changes in the upper œsophagus are of a more complex nature than in other regions and that this complexity seems in general to favor deviations in development. This may in part account for the gland areas in this region and also for their considerable individual variations.

Schaffer has compared the superficial glands to the œsophageal glands of some of the lower animals. Yet, if we regard the presence of parietal cells as an important feature in these glands, they have no homologue in any described structure in lower animals. The œsophageal glands of birds are above the muscularis mucosæ and in this respect they resemble the superficial glands of man. On the other hand, they are definite mucous glands and show no tendency to occur in such definite paired areas as do the superficial œsophageal glands.

The pathological relations of these glands are of interest mainly on account of their possibilities. It has been suggested that being a place of lessened resistance in the œsophageal wall a large area might gradually give way and so become the starting point for a pulsion diverticulum. The chief support for this interesting speculation comes from the fact that both the pulsion diverticula and the superficial gland areas are situated in the same region of the œsophagus. That the strength of the mucous membrane of the œsophagus is a

very important factor in the resistance to dilatation does not seem probable a priori. Should a large gland area be the point of origin of the diverticulum one would expect to find columnar epithelium in the walls of the diverticulum corresponding to the original area of superficial glands. Of special interest in this regard is König's[14] observation that two of his diverticula contained both cylindrical and flat epithelium. This seems to be the only observation of this kind on record. In a case recently in Dr. Halsted's service in the Johns Hopkins Hospital the wall of the diverticulum was lined uniformly by a pale mucous membrane which proved on section to be covered by stratified epithelium. There was no evidence of the presence of a superficial gland area.

That the glands may be a source of origin for œsophageal cysts seems probable from the fact that they commonly contain numbers of small retention cysts. The largest one in my specimens (0.7 mm.) is just visible to the unaided eye. Ordinarily these small cysts probably cease to grow larger or if they do so they are especially liable to rupture. In a few cases, however, they doubtless continue to enlarge and so give rise to one form of cysts in the œsophageal wall.

The most interesting and important question deals with the possibility of the superficial glands giving rise to carcinomata. Schaffer and Hildebrand both emphasize this possibility. I have collected from the records six cases of adeno-carcinoma of the œsophagus where the locations are mentioned, in order to see if these locations bore any relation to the location of the superficial glands. Of these carcinomata, two[15] were situated somewhat above the middle of the œsophagus, two[16] were somewhat below the middle, and two[17] more

[14] Die Extirpation des Oesophagusdivertikel. *Berl. klin. Wochenschr.*, 1894, xxxi, p. 948.

[15] F. Colle, Beiträge zur Lehre vom primären Oesophaguscarcinom. Inaug.-Diss. Göttingen, 1887.

O. Fischer, Ueber einen Fall von primärem Carcinoma myxomatodes des Oesophagus. *Prager med. Wochenschr.*, 1899, p. 391.

[16] Parmentier, *Arch. gén. de méd.*, 1889, i, p. 470.

Karewsky, Carcinom des Oesophagus. *Deutsche med. Wochenschr.*, 1892, p. 1070.

[17] D. Newman, Malignant Disease of the Throat and Nose. Edinburgh and London, 1892; cited from Rolleston in Clifford Allbutt's System of Medicine, iv, p. 374, New York, 1897.

C. P. White, *Trans. Path. Soc., London*, 1898, xlix, p. 93.

were immediately above the cardiac orifice, yet distinctly separated from the stomach. It will be seen that none of these adenocarcinomata lies within the usual limits for the superficial glands in the upper œsophagus, although the last two lie in the region of the lower superficial glands (Cardiadrüsen). The first four lie in a region where superficial glands are comparatively rare.

That the superficial glands are totally distinct from the submucous glands is easily seen where they are so well developed as in our first specimen. When they are less numerous, however, and are covered by stratified epithelium, recognition is not so easy. The most constant and important differential point is, as we have said, the relation to the muscularis mucosæ, probably all glands completely above this being superficial glands. The superficial glands tend to occur in groups in definite regions of the œsophagus. The mucous glands are generally distributed over the whole œsophagus and lie in the submucosa. The ducts of the submucous glands are lined by stratified cuboidal epithelium; the ducts of the superficial glands by a single layer of columnar cells. Lymph nodules lie about the ducts of the submucous but are beneath the bases of the superficial glands. The superficial glands frequently contain parietal cells, while the submucous glands never do. The mucous glands take a deep mucin stain; the superficial glands take it but slightly. These characteristics show that the superficial œsophageal glands are quite distinct from the submucous glands and, as a rule, are readily recognizable.

In conclusion I desire to thank Dr. Ophüls for his kind permission to report this interesting case of well developed superficial œsophageal glands, as well as for his assistance and the many favors he has shown me during my work in his laboratory.

DESCRIPTION OF PLATE XXI.

Fig. 1.—Photograph of gross specimen which had been preserved in Kaiserling's fluid. The œsophagus is opened along its left side and is turned over to the right. One of the two superficial gland areas is seen as a dark diamond-shaped area on the surface of the œsophagus. The corresponding area of the left side was removed for histological examination.

Fig. 2.—Photomicrograph of a section passing through the edge of the left superficial gland area. Hæmatoxylin and eosin stain. x 17. Above and to the

FIG. 1.

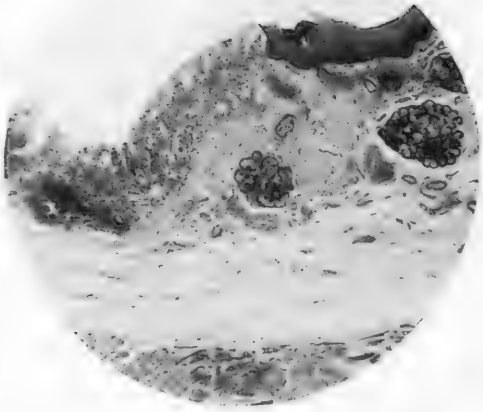

FIG. 2.

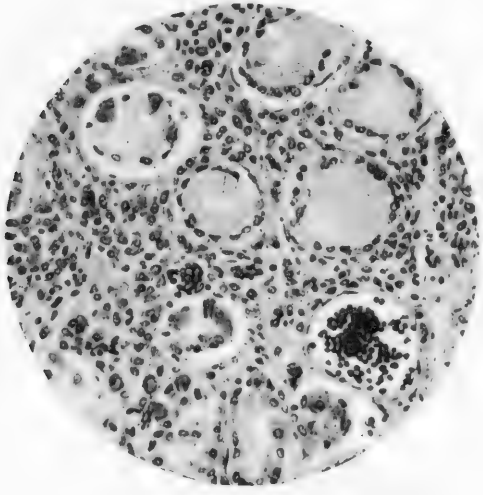

FIG. 3.

right the squamous epithelium is seen, passing toward the left, where it gives place abruptly to the superficial glands. The darkly stained, irregular ovoid masses below the muscularis mucosæ are the submucous glands with the mucus stained by the hæmatoxylin. Their ducts can be seen above them. The muscularis mucosæ is seen superficial to the submucous glands on the right. At the border of the superficial glands it is much thickened. To the left it is very thin and applied closely to the bases of the superficial glands. A lymph nodule is seen on the left just above the muscularis mucosæ. Striated muscle of the upper œsophagus is seen below.

Fig. 3.—Photomicrograph of a section near the bases of the superficial glands, x 220. Stained with Heidenhain's iron hæmatoxylin. Red blood corpuscles are deep black. Near the centre are several acini containing no parietal cells. Acini in the upper left and the lower parts of the figure contain many parietal cells.

FALSE DIVERTICULA OF THE INTESTINE.*

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PLATES XXII–XXVI.

Diverticula of the intestine may be divided into the true and the false. By a true diverticulum is understood one the walls of which are composed of the entire thickness of the intestine; the false diverticulum, on the other hand, is a hernia of the mucosa and submucosa through the muscular wall (von Rokitansky (1), Leichtenstern (2), Birch-Hirschfeld (3), Orth (34), Hansemann (4), and others). Therefore, while a true diverticulum presents all three coats of the gut, a false diverticulum is formed of mucosa and serosa only, with intervening connective tissue. It is further desirable to differentiate between congenital and acquired diverticula. This distinction, however, does not apply to false diverticula, as, so far as known, all of these are acquired, no case of congenital false diverticulum of the intestine having ever been described.

Edel (5) and others would make synonymous the terms congenital diverticula and true diverticula, likewise the terms acquired diverticula and false diverticula, specifying that the former have all three coats of the intestinal wall, while the latter show only mucosa and serosa. The difficulty in determining definitely in many cases whether the sacculation is congenital or acquired, makes this classification objectionable. A definition based upon morphological rather than developmental conditions, though less desirable, is at least a safe one. Furthermore, Edel's definition does not hold good in all instances, as cases of acquired diverticula possessing all coats of the intestine have repeatedly been reported. Thus Fiedler (6) records such cases. Wallmann (7) has described nine true acquired diverticula of the large intestine. Norman Moore (8) has reported a case of multiple diverticula of the small intestine in a man

* Read before the Chicago Pathological Society, November 10, 1899.

40 years old. The diverticula occurred at the mesenteric attachment of the gut, and were composed of all coats of the intestine. Moore does not state whether he regarded the diverticula as congenital or acquired.

By far the greater number of true intestinal diverticula of congenital origin are the Meckel diverticula, which are formed by a persistence of the omphalomesenteric duct. Other factors may, however, give rise to such diverticula. Grawitz (9) has found a diverticulum of the large intestine whose origin could be traced to fetal malformation. Buchwald with Janicke (10) has described a true diverticulum of congenital origin in the jejunum of a boy 6 years old.

Hansemann (4), Neumann (11), and Nauwerck (12) have each reported a case of true intestinal diverticula with accessory pancreas at their apices. Hansemann's case was of a diverticulum at the upper end of the jejunum of a boy 14 years of age. Neumann found a true diverticulum with a pea-sized accessory pancreas at its apex, a case similar to that of Zenker (13), who found, 54 cm. above the ileocæcal valve, a diverticulum at the apex of which was a cherry-sized pancreas. Nauwerck's diverticulum came from the ileum of a man 43 years old and possessed a separate mesentery. Except Zenker, who interprets his case as one of true Meckel's diverticulum, the observers all attribute the occurrence of the intestinal sacculations to traction brought about by the accessory pancreas. Other instances, in which traction has been believed to be the causative agent, have been described by Birch-Hirschfeld (14). These were caused by connective-tissue bands in the mesentery. In two cases described by Hansemann (4), a tumor produced the traction.

The first description of false diverticula of the intestine is usually attributed to Sömmering (37) in his translation into German of Matthew Baillie's "Morbid Anatomy" (1794), but Voigtel (38) cites previous cases recorded by Schröck, Riolan, Günz, Morgagni and Haller, at least some of which undoubtedly belong to this class. The subject is considered in the text-books on pathological anatomy of Cruveilhier, Rokitansky (15), Förster (33), Orth (34), and others.

Fleischmann (16) described in 1815 a false diverticulum of the duodenum. Roth (17) in 1872 gave a critical summary of the cases of duodenal diverticula reported up to his time, and described five personal observations. He came to the following conclusions: Diverticula of the duodenum are exclusively of the false variety; usually only one or two, less frequently three or four are present; they do not become larger than a pigeon's egg, and are usually empty; they occur most commonly at the inner, posterior aspect of the pars descendens duodeni at the entrance of the biliary and pancreatic ducts.

Wallmann (7) found in a part of the small intestine, 48 cm. long, thirty-seven diverticula, of which thirty were said to be between the folds of the mesentery. The diverticula were false, the walls being composed only of the mucosa and serosa. Birch-Hirschfeld (3) has described a case in which the ileum through almost its entire length showed many false diverticula along the mesenteric border. Klebs (18) found in the small intestine of an old man twenty diverticula, varying in size from a pea to a walnut, and all situated within the mesentery. Bristowe (19) found just above the ileocæcal valve a solitary diverticulum, the walls of which were formed of mucosa and serosa.

False diverticula of the large intestine have repeatedly been described. Gross (20) described and pictured this condition. Bristowe reported a case of diverticula of the large bowel occurring in a woman 69 years old. The walls of the sacculations were formed of mucosa and serosa only. The diverticula were limited to the site of the appendices epiploicæ, and varied in size from a pin's head to a marble. Alibert found two hundred diverticula in a colon, while other instances of false diverticula of the large intestine have been described by Schröder, Sydney Jones, Astley Cooper, and others.

All writers, with the exception of Förster,* agree that the false diverticula of the small intestine occur almost exclusively on the concave side of the gut, and that they lie between or close to the layers of the mesentery. Their average size varies from that of a pea to that of a pigeon's egg, but Virchow (21) has shown a specimen in which the diverticula attained the size of a hen's egg.

The etiology of false diverticula is still somewhat obscure. Reference has been made to the production of diverticula by traction; this, however, is not generally believed to be the usual cause. It was for a long time held that they were due to increased intra-intestinal pressure of various kinds, as from fæces, or gas. Klebs (22) was the first to point out that a certain relation exists between the blood-vessels and the diverticula. In his case already cited, the small sacs were true, the larger, false diverticula, and all were at the points of entrance of the mesenteric vessels. This condition led Klebs to the suggestion that either through growth of the mesentery in length outstripping

* As suggested by Hansemann, Förster's apparently exceptional position may be the result of a typographical error, as his description otherwise corresponds to that usually accepted.

that of the blood-vessels, or more probably through traction in later life upon the mesentery by the intestine, the funnel-like attachment of the intestinal wall to the entering mesenteric vessels becomes converted into a diverticulum. In support of the latter supposition is the greater frequency of these multiple diverticula in elderly and obese persons.

While Good (23) and Hanau (24), and Hansemann (4) confirm Klebs's observation concerning the relationship of false diverticula of the small intestine to the blood-vessels, they attribute the origin of the diverticula to pulsion rather than to traction, and Hansemann has defined more precisely the vascular relationship. Intestinal diverticula occur in those who at no time have given indications of any notable deposit of fat in the mesentery. In a case reported by Hansemann the patient, who died of pneumonia, was 85 years old and emaciated, having always been thin. About 400 diverticula, varying in size from a millet-seed to a pigeon's egg, were counted, the larger number being in the jejunum and upper part of the ileum. There were some, however, in the duodenum and many in the sigmoid flexure, but none in the vermiform appendix or colon. Those in the small intestine were mostly empty, but some of those in the large intestine contained fæces. Most of the diverticula occurred close to the mesenteric border, but not actually between the layers of the mesentery. Some in the large bowel were on the convex side of the gut. An interesting relation existed between the diverticula situated at the mesenteric border and the blood-vessels; the vessels branched out in the diverticular walls, those beneath the serous surface being arteries, while those visible from the mucous surface were veins. The diverticula had no muscular tunic. The fact that the mucosal hernias were not separated from their corresponding veins led Hansemann to conclude that the false diverticula were formed by the bulging of the mucosa through the muscularis along the sheaths of veins passing from the intestine into the mesentery.

Heschl (25), in 1880, showed that the intestine ruptures under pressure at the points where the diverticula are found. Good, working under Hanau's direction, by filling a loop of intestine with water and

subjecting it to hydraulic pressure, was able to produce trench-like furrows along its mesenteric border before perforation occurred. Hansemann was more successful, as he was able by a similar procedure to produce in the small intestine sacculations which were far more typical than any produced by previous experimenters, and to demonstrate that these bore the same relation to the veins as the false diverticula observed in his patient.

Senility doubtless favors the formation of intestinal diverticula, as this lesion is very rarely found in young persons. Hansemann's attempts to produce diverticula experimentally succeeded when senile intestines were employed, but failed entirely with the intestines of children.

Besides the normal loci minoris resistentiæ, other etiological conditions are to be considered. Abnormally great intra-intestinal pressure loses some of its importance as a causative agent when it is remembered how rarely post-mortem examination reveals diverticula of intestines in which such pressure has undoubtedly been present. Chronic constipation may be considered as a predisposing factor, especially in those true diverticula of the colon which represent scarcely more than a dilatation or exaggeration of the normal haustra coli, but here also there is marked discrepancy between the frequency of habitual constipation and the occurrence of diverticula.

Fleischmann pointed out that the muscular fibres of the duodenum are normally separated by the common bile duct, thus furnishing a condition favorable to the formation of a diverticulum in this part of the duodenum. Roth mentioned fatty degeneration of the muscularis as a possible cause. A stenosis, leading to increased intestinal pressure, may be produced by large gall-stones retained within the intestine. Instances of diverticula from this cause are cited by Leichtenstern (2). A retention cyst of the appendix may lead to the production of diverticula in it (Kelynack (26), Ribbert (27)). Finally, attention may be called to the influence, predisposing to diverticular formations, of general sluggishness of the intestinal functions, due to marasmus, phthisis, or carcinoma.

The existence of intestinal diverticula is usually unattended by

clinical symptoms, the presence of the sacculations being discovered only upon post-mortem examination. Sometimes, however, serious pathological processes ensue, as in Sydney Jones's (28) patient, who passed fæces in the urine, in consequence of ulceration of the end of a diverticulum of the sigmoid flexure into the bladder. Alfred Loomis (29) found in the sigmoid flexure and colon descendens of a man 61 years old many highly inflamed diverticula filled with fæces. Although no perforation had occurred, the inflamed diverticula had originated a general peritonitis. Two other cases of peritonitis, associated, however, with perforation, have been described by Fiedler. Klebs (30) has described a case of hernia obturatoria in which a diverticulum had perforated into the hernial sac. One of the favorable terminations of diverticula is, according to Fano (31), obliteration through an increase in the connective-tissue elements. This is quite frequent in congenital but rare in acquired diverticula.

Case I.—This is a museum specimen of a piece of jejunum 7½ cm. long, in the centre of which is a small, oval diverticulum. No other diverticula were found in the intestinal tract. The peritoneum is smooth. The diverticulum is as large as a bean, and lies beneath the peritoneum of the mesentery at one side of the line of mesenteric attachment. Blood-vessels run over its serous covering (Plate XXII, Fig. 1). On the intestinal aspect the valvulæ conniventes are arranged in the normal way. An opening, which admits a small-sized probe, marks the point of communication between the intestinal canal and the diverticulum. This opening is very much smaller in size than the diameter of the saccule, and is surrounded by the valvulæ conniventes, three of which dip down into the mouth of the diverticulum, thereby almost closing the opening (Plate XXII, Fig. 2).

Microscopic examination: The diverticular walls are composed of mucosa, submucosa and serosa only. The muscularis stops abruptly at each side of the sac, where it is bunched as though the out-pouching mucous membrane had crowded it aside. The cells of the mucosa are very granular, and their nuclei stain but faintly. The lower portion of the mucosal hernia is highly inflamed. The submucosa is recognizable with difficulty. The muscularis shows faintly stained nuclei, but otherwise no change. The serosa is normal.

Case II.—Emma T., aged 59, a housewife, was admitted to the hospital suffering from pulmonary phthisis and interstitial nephritis. The

autopsy, made by Dr. Hektoen, showed the body of a slender, emaciated woman. The anatomical diagnosis reads: False diverticula of the ileum, chronic pulmonary tuberculosis, chronic nephritis, chronic splenitis, atheroma of the aorta, atrophic liver, general marasmus.

The peritoneum was empty; the omentum adhered to the anterior abdominal wall. The mesentery was of full length. The lower part of the ileum was rather small in calibre; in the first metre and a half were numerous large and small, variously shaped diverticula. The large intestine was normal.

The diverticula are scattered along the mesenteric attachment of the ileum. They vary greatly in size, some being no larger than a split pea, while others are fully half the diameter of the intestine. The larger ones are often lobulated, so that a large diverticulum may present one or more smaller ones upon its surface. The sacculations are covered by peritoneum, which has often been loosened from its intestinal attachment by the out-pouching diverticulum. The larger diverticula show, for the most part, greatly constricted bases, while the smaller ones have broad and expanded ones. The diverticula are not situated exactly between the two mesenteric layers, but usually show a greater bulging on one or the other side of the mesentery (Plate XXII, Fig. 3).

There is an undoubted connection between the blood-vessels and the diverticula. The larger diverticula have the blood-vessels running over their surfaces; the smallest diverticula look as though blood-vessels pierced their apices; at other times, the blood-vessels run over their surfaces (Plate XXII, Fig. 3).

Microscopic examination: All of the diverticula are false, their walls being composed only of mucosa, submucosa, and serosa. The muscularis is separated, the mucosa pushing through it to unite with the serosa and form the saccule (Plate XXIII, Figs. 4 and 5, and Plate XXIV, Fig. 6). The mucosa of the diverticula is faintly stained, granular, and atrophic. In the smaller diverticula it is markedly infiltrated with leucocytes. This inflammation is most marked at the apices of the mucosal hernias, and is most intense in the deeper parts of the mucous membrane. The muscular coat is lacking over the greater part of the diverticulum. On either side of the sac the musculature presents the normal appearance. In some of the specimens the muscular layer shows a sort of club-shaped thickening at the point where the diverticulum begins; sometimes a thin strip of muscular tissue is seen running a part of the way over the wall of the diverticulum at the sides (Plate XXIII, Figs. 4 and 5). When the latter occurs, the muscle thins out gradually until it finally loses itself in the sub-serous connective tissue. The serosa

shows marked increase of connective tissue and often a small amount of round-cell infiltration. In the smaller diverticula a large blood-vessel is usually found at the apex of the saccule. Sometimes more are found, depending in part upon the size of the diverticulum. All these vessels show much thickening of their walls. In no instance have I been able to find intestinal musculature about the blood-vessels of the diverticula. Observations concerning the early development of the diverticula in this case are considered below (p. 343).

Case III.—This specimen of false diverticula of the rectum, sigmoid flexure, and descending colon, was kindly given me by Dr. Le Count.

William P., aged 64, laborer, was admitted to St. Elizabeth's Hospital complaining of pain across the stomach and in the right iliac fossa. The post-mortem examination made by Dr. Le Count gave the following anatomical diagnosis: Diverticula of the rectum, sigmoid flexure, and descending colon; hypertrophy of the heart; general arterio-sclerosis; chronic passive hyperæmia of the liver, spleen, kidneys and lung; œdema of the lower extremities; Meckel's diverticulum; right-sided obliterative pleuritis; slight chronic nephritis; œdema of the brain.

There are adhesions about the splenic flexure of the colon and about the sigmoid flexure. The mucosa of the large intestine is everywhere smooth. In the rectum, sigmoid flexure, and descending colon are found about twenty-five diverticula. The greater number of these are in the rectum and lower portion of the sigmoid. No diverticula are found above the descending colon. They are situated near the mesenteric attachment of the bowel and are from one-half to two centimetres in diameter externally. They differ somewhat in appearance, the smallest appearing as mere digital depressions in the mucosa. The diameter of the orifices is less than that of the saccules with which they communicate. In the larger diverticula labia of mucous membrane surround the openings. The walls of none have perforated, though some are extremely thin. An undoubted relation exists between the diverticula and the blood-vessels. By holding the bowel to the light the vessels can be seen to enter the summit of the diverticula and then, branching, to run over their surfaces (Plate XXIV, Fig. 7).

Microscopic examination: The diverticula are of the false variety. The walls of the larger diverticula are composed of mucosa and serosa only, with intervening connective tissue, but the smaller, beginning diverticula show a muscular wall which has undergone thinning to a greater or less degree. The mucosa of the intestine in general shows no change, but the changes in the mucous membrane of the diverticula are in some places well marked. These consist of a granular disintegra-

tion of the cells associated with loss of nuclei, and an infiltration of the mucosa and submucosa in places with leucocytes. This evidence of inflammation is often most marked in the deeper portions of the diverticula, where, owing to the small diameter of the orifice, communication with the interior of the intestine is difficult. This case was found to be particularly suitable for the study of the early stages of development of diverticula and the results obtained are presented below (pages 344-347).

Case IV.—This example of diverticulum of the appendix was found in the dissecting room of Rush Medical College. I have to thank Dr. Parker for bringing the case to my notice, and Mr. F. M. Wood for details of the pathological findings.

The specimen came from the body of a man about 50 years old, with pulmonary tuberculosis and general arterio-sclerosis. No history could be obtained. The vermiform appendix, bound down by firm adhesions, sprang from the lower and posterior part of the cæcum, running inward, downward and backward so as to lie over the pelvic brim. It is 7½ cm. long and 7 mm. in diameter in its thickest part. It possesses a separate mesentery in which are found blood-vessels and strands of connective tissue. Six centimetres above the tip of the appendix, on the side opposite the mesentery, is a sacculation measuring 12 x 11 mm., with a somewhat constricted base (Plate XXIV, Fig. 8). A few small blood-vessels are seen running over its surface. A little below the site of the saccule, toward the distal extremity, a constriction extends half around the appendix, causing a diminution in its calibre. The appendix, with the diverticulum on the external aspect, is covered by shreds of torn fibrous adhesions. The mouth of the appendix is patulous. By probing, it is found that the lumen is patent only from the mouth to the constriction; beyond this point it is obliterated by connective tissue. The diverticulum and the patulous lumen of the appendix are filled with soft fæcal matter in which are found several hard, irregularly shaped, calcareous granules, varying in size from grains of sand to fine gravel. The mucous lining of the patulous lumen is smooth; the wall of the appendix is thickened. The wall of the diverticulum is no thicker than tissue paper; its lining is slightly roughened.

Microscopic examination: The obliterated part of the appendix shows the lumen to be entirely filled up by a loose network of connective tissue; no evidences of mucosa are visible. External to the loose connective tissue is a ring of dense mature connective tissue entirely encircling the appendix. This is evidently the much thickened submucosa. The connective tissue in both these places shows many newly-formed blood-ves-

sels. The muscularis consists of a thick internal circular and a thick external longitudinal coat. No degenerative changes are visible in either layer. The serosa is thickened and presents, at the points corresponding to the torn adhesions, a ragged peritoneal edge. None of the normal lymph follicles are present, and no evidences of an acute inflammatory condition can be found. At the constriction below the diverticulum there is entire loss of the muscular coat, the wall of the appendix here being made up of the connective tissue of the submucosa and the slightly thickened serosa (Plate XXV, Fig. 9). The mucosa over the constriction and extending above and below it, is highly atrophic, remnants only of the crypts being left; at the site of the constriction the mucosa is covered by a thin layer of deeply stained, granular detritus. The submucous connective tissue is greatly thickened and here there are many well-filled, newly-formed blood-vessels; many large empty spaces are present between the strands of connective tissue. No lymph follicles are seen. The muscularis stops abruptly at the lower side of the constriction and more gradually at its upper; the internal layer is thick, the external somewhat thinned. The connective tissue of the serosa is slightly thickened.

The mucosa opposite the large diverticulum is represented by the remains of a few crypts. The enormously thickened submucosa here constitutes the greater part of the wall. It is not inflamed, but many well-filled blood-vessels are seen. Both muscular layers are much thinner than in the sections already described. The serosa is somewhat thickened, so much so at one point that a wedge-like mass of connective tissue has taken the place of the outer muscular coat. The fibrous tissue shows outward pouching.

The wall of the diverticulum is everywhere extremely thin (Plate XXV, Fig. 10). The mucosa of the appendix becomes gradually lost as it merges into the diverticular lining, which is represented by a deeply stained blue line of fæcal matter, no actual mucosa being visible. The wall is composed only of submucosa and serosa. The muscularis is gradually lost at the point where the diverticulum begins (Plate XXV, Fig. 10). Many large blood-vessels are found in the wall of the diverticulum. The peritoneal surface shows many torn strands of connective tissue, but no changes of an acute inflammatory nature are seen.

Case I, in being an example of a single diverticulum of the jejunum, contrasts with the cases of jejunal diverticula reported by Virchow (21), Edel (5), and Nicholls (32), which were all of multiple diverti-

cula, such multiplicity being the rule with this type of diverticulum. Roth (17) and others have recorded instances of a single diverticulum of the duodenum, and Bristowe (19) one of a solitary diverticulum of the ileum. The gross and microscopic appearances of the diverticulum in Case I were those characteristic of false diverticula. The intimate relation to the blood-vessels is to be emphasized. The marked inflammation of the mucous lining of the sac is an interesting feature.

Case II is similar to those already cited from Wallmann, Birch-Hirschfeld, Hansemann and Klebs. Hansemann has called attention to the fact that the diverticula in his case did not go directly into the mesentery but lay to one side of it. This same condition has prevailed in both my cases. The tunicary hernia of the jejunum, though covered on all sides by peritoneum, still lay entirely on one side of the mesentery; likewise in the diverticula of the ileum, though some lay just between the mesenteric leaves, the majority showed most prominently on one side of the line of attachment of the mesentery.

My case of diverticula of the ileum is interesting inasmuch as it shows clearly the intimate connection between the blood-vessels and the sacculations. A systematic study of the variously sized diverticula has shown the following points:

The smallest diverticula which could be found—diverticula macroscopically about the size of a split pea—show within the serosa a blood-vessel with much thickened walls. On either side of this vessel may be seen a beginning down-dropping and protrusion of the mucosa. This gives the mucosa a W-shaped appearance, with the blood-vessel contained between the two middle arms (Plate XXIII, Fig. 4). Diverticula somewhat larger show a greater protrusion of the mucosa and an outward movement of the blood-vessel at the apex of the saccule (Plate XXIII, Fig. 5). Thus the blood-vessel is borne outward by the out-pouching diverticulum. At this stage of development, it seems that one side of the saccule may grow faster than the other, so that ultimately the blood-vessel is seen to lie at one side of the diverticulum (Plate XXIV, Fig. 6).

This manner of development indicates that the location of a large blood-vessel in a certain place in the muscularis of the intestine pro-

duces here a locus minoris resistentiæ; a causative agency of some kind produces an internal pressure which manifests itself by a hernia of the mucosa at the point of least resistance; as no muscular tissue of the intestine is ever found to surround the blood-vessel of the diverticulum, it seems reasonable to suppose that the false diverticulum is formed by a protrusion of the mucosa along the sheaths of the blood-vessels.

Conditions favorable to diverticular formation were present in this case. There was a general marantic condition with chronic pulmonary tuberculosis and chronic nephritis; the intestine, through its entire extent, was thin and flabby; the lower part of the ileum was of small calibre, a condition which, if not due solely to agonal contraction, might at least favor the production of diverticula in the upper part. The inflammatory condition of the mucosa and serosa is also worthy of note.

The detailed study of the process of mucosal hernia through the muscular coat is not so simple as might at first seem to be the case. In the two cases of diverticula of the small intestine above described the thinness of the muscular coat prevented a careful study of the part played by each of the layers, but the thickness of the muscular sheath of the sigmoid and rectum permitted me to study in Case III to better advantage the participation of each in the production of the diverticulum.

As the studies of Hansemann (4) and Graser (35) have shown, the veins draining the intestine pierce the inner muscular layer, run between the two muscular layers, and finally pierce the outer longitudinal layer to make their appearance externally. Graser finds, as previously Hansemann had done, that false diverticula follow the venous sheaths. His view concerning their formation in the sigmoid flexure, to which his studies were limited, is briefly this: a congestion of the veins of the sigmoid brings about dilatation of these vessels, causing thereby a locus minoris resistentiæ in the muscular tunic of the intestine; an intra-intestinal pressure such as the presence of gas or fæces furnishes the initiative force required to produce the diverticula at these points of least resistance.

My case (Case III) of diverticula of the rectum and sigmoid flexure corroborates to a great extent the views advanced by Graser. The blood-vessels throughout the specimens examined showed marked congestion, being very large and filled with blood, and there was little difficulty in tracing the connection between the diverticula and the distended blood-vessels. The smallest diverticulum that I was able to find—one that showed macroscopically as a slight digital depression in the mucous membrane—showed the beginning stages of the mucosal hernia. Corresponding to the slight invagination of the mucosa, within the inner layer of the muscular tunic was found a large congested blood-vessel; on either side of this a well-marked thinning of the muscular sheath had already occurred; this thinning was most evident in the inner muscular layer, the outer layer being still intact (Plate XXVI, Fig. 11). A somewhat larger diverticulum showed on histological examination a greater depression of the mucosa with a greater thinning of the inner muscular layer (Plate XXVI, Fig. 12). For some time at least, the outer longitudinal muscular layer may remain intact, being pushed more and more outward by the protruding mucosa (Plate XXVI, Fig. 13). As the diverticulum continues to grow the muscular layer surrounding it becomes very much thinned and finally disappears entirely, so that the wall of the diverticulum is ultimately composed of mucosa, submucosa, and serosa only.

The foregoing description does not, however, apply to all the diverticula. Although the inner, circular coat usually seems to be the one to give way first, it sometimes happens that the outer shows the first break in its continuity. It would seem that in these cases the blood-vessel had occupied the outer layer of the muscular tunic, in this way changing the locus minoris resistentiæ from the inner to the outer layer of the muscularis. Thus such conditions as I have pictured in Fig. 14 (Plate XXVI) come to pass, in which the mucosa and the inner muscular layer rupture through the outer one. In this process the blood-vessel is evidently pushed ahead of the oncoming diverticulum.

Finally, it would seem that under some conditions a break occurs simultaneously in both layers of the muscular sheath. This occurs in those places where several large vessels are situated closely together,

so that the entire muscular wall is so weakened that the mucous membrane breaks through both with comparative ease (Plate XXVI, Fig. 15).

It is now the generally accepted belief that the diverticula follow the paths of the venous sheaths (Plate XXVI, Figs. 16-17); still, though this be in the main correct, I cannot accept it in its entirety. Although the presence of the blood-vessel is the prime, predisposing cause leading to the production of the mucosal hernia, still, once started, the saccule follows the path of least resistance. This may or may not be the sheath of the blood-vessel. Instead of following the latter, the mucous membrane may spread between the layers of the muscular tunic (Plate XXVI, Fig. 13), or it may spread through the connective tissue between the muscularis and the serosa in places where it is impossible to establish any connection between the diverticulum and the blood-vessels.

The partially successful effort of nature to limit the enlargement of the diverticula is evidenced by the connective-tissue hyperplasia about them. The thickness of the serosa covering the diverticula is usually almost directly proportional to their size; and there can be no doubt that to this protective attempt of nature is due the infrequency of perforation of diverticula into the peritoneal cavity.

Doubtless an important factor in the causation of the diverticula in Case III was the chronic passive congestion of the viscera due to hypertrophy and dilatation of the heart and arterio-sclerosis. While this factor has not the exclusive importance attached to it by Graser, it is certainly one of the demonstrable predisposing causes of intestinal diverticula. Constipation furnished another condition favorable to the process by increasing the intra-intestinal pressure.

The presence in this case of firm adhesions about the rectum and sigmoid, corresponding to the position of the diverticula, is also of interest. As has been stated, some of the diverticula were highly inflamed. It can readily be understood how easily these pockets, communicating as they do through only small openings with the lumen of the intestine, might harbor fæcal matter, the accumulation of which might lead to an inflammatory condition. How difficult the removal

of such material may be, is evident from Fig. 13 (Plate XXVI), which shows a section through one of these highly inflamed diverticula. Graser has pointed out the relation which may exist between diverticula of the lower bowel and chronic pelvic peritonitis. I interpret the adhesions which were formed about the sigmoid flexure in my case as an evidence of such a chronic pelvic peritonitis engendered by the inflammatory condition of the diverticula. Further evidence of such a peritonitis is found in the pain in the iliac fossa of which the patient complained. A few diverticula were found in the region of the beginning descending colon, and here, too, slight adhesions had formed.

Case IV is an example of diverticulum of the vermiform appendix —a condition concerning which but little has been written. Finkelstein (36) has suggested the possibility of the production of a diverticulum from increased intra-appendicular pressure following occlusion of the mouth of the appendix and consequent collection of secretion. Two cases have been recorded. That of Kelynack (26) was of an appendix which became cystic in consequence of obstruction to its opening. Two very distinct diverticula communicating with the lumen of the appendix were found between the folds of its mesentery. No muscular tissue was detected in the walls of the diverticula and hence the conclusion seems justifiable that they were mucosal hernias through the muscularis in consequence of the pressure produced by the stasis of the secretions within the appendix.

In Ribbert's (27) case there was a group of small cysts of varying size, arranged in a grape-like cluster at the mesenteric border of an apple-sized cyst of the appendix and communicating with the latter through larger and smaller openings. The large cyst was the result of occlusion of the mouth of the appendix. The small cysts are interpreted by Ribbert as dilated glandular cysts which had become partly occluded and separated from the mucosa. If this interpretation be correct, these cysts would not be analogous to the ordinary false diverticula of the intestine.

In some respects, more like my case is the one reported by Edel (5), who found in a man 69 years of age a pea-sized false diverticulum, situated on the side opposite the mesentery, 2 cm. from the distal end

of the appendix, which was 8 cm. in length. A small blood-vessel ran over its surface. There were no fæcal contents in the appendix or diverticulum, and its lumen was patent. The muscularis was absent over the diverticulum. The mucous membrane was atrophic; the serosa showed many new blood-vessels. By the side of the diverticulum the muscle was replaced by a wedge-shaped mass of connective tissue, which, though of unknown origin, was thought to represent a scar. For the formation of the diverticulum in this case Edel offers the following explanation: the scar produced a slight traction upon the mucosa and, as the result of connective-tissue increase in the serosa, the latter began to bulge out. Assuming that the traction upon the mucosa increased, and that the muscularis finally yielded to the slight but continued pressure of the mucosa, he was led to believe that the production of a false diverticulum thereby resulted.

I am unable to say what caused in my case the mass of adhesions found about the appendix and its diverticulum. A tubercular or syphilitic etiology is possible. There were no acute inflammatory lesions, and in the absence of a history we must leave this point unexplained. If we believe with Ribbert that obliteration of the appendix represents a process of involution and not one of pathological change, then little is to be said concerning the obliteration of the lower part of the appendix in my case. Loss of lymph follicles and replacement with hyperplastic connective tissue are both found, so that all the conditions described by Ribbert as characteristic of this type of obliteration are represented.

The constriction of the appendix on the distal side of the diverticulum is interesting in so far as it presents the typical structure of a false diverticulum without a bulging of the weakened part. The wall of the appendix at the point of the constriction is covered with a mucosa upon which lies a deeply stained granular detritus. Longitudinal serial sections passing from the non-constricted into the constricted portion of the appendicular wall show a progressive thinning of the muscular coat, ending finally in its total loss (Plate XXV, Fig. 9). Under ordinary conditions, we would expect the mucosa to bulge outward as the muscular coat became thinner, still in this case, the oppo-

site has occurred and the serosa has fallen in toward the mucosa. This seems to me to be a natural sequence when we remember that the connective tissue of the submucosa is fully four times as thick as, and hence, firmer and more unyielding than the connective tissue of the serosa. Thus, instead of the formation of a diverticulum, a constriction has resulted.

The cause of the production of the large diverticulum can at best only be surmised. As in Edel's case, it occurred on the side opposite the mesentery, so that its formation as a mucosal hernia along a vascular sheath is extremely unlikely. It may be recalled that the appendix was embedded in firm adhesions; that the diverticulum was filled with fæcal matter; and that the wall was composed of thickened serosa only. True intestinal diverticula due to traction produced by an accessory pancreas, or by connective-tissue bands in the mesentery have several times been described. It seems to me not impossible that in the beginning this diverticulum of the appendix was a true one, due to the traction of a connective-tissue band, and that this mural bulging became filled with fæcal matter, the accumulation and stagnation of which caused a pressure atrophy of the mucosa and muscularis of the diverticulum. Thus the wall of the diverticulum became weakened and composed of connective tissue only.

As we have no means of learning whether the adhesions antedated the diverticulum, another explanation may also be suggested. It may be that in consequence of the pressure and irritation of fæces accumulated in the appendix the mucosa first and then the muscularis underwent necrosis or simply atrophy. There would thus be produced in the wall of the appendix that which is essential to the formation of a diverticulum in any part of the intestinal tract, namely, a point of lessened resistance, which, under the further influence of an increased intra-intestinal pressure, as from meteorism or constipation, might readily be the starting point of a diverticulum presenting the histological features observed.

The possibility may also be considered that the same process, whether pathological or referable to simple involution, which had caused the constriction, may have affected a limited area in the wall

of the appendix causing a focus of lessened resistance, which yielded to the normal or perhaps increased pressure within the lumen of the appendix, before any considerable compensatory new connective tissue had formed.

I am indebted to Professor Hektoen for much kind assistance in this investigation and in the preparation of this paper.

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DESCRIPTION OF PLATES XXII-XXVI.

PLATE XXII.

Fig. 1.—Case I. Jejunum, showing the relation between the blood-vessels and diverticulum.

Fig. 2.—Case I. Arrangement of the valvulæ conniventes of the jejunum about the diverticulum.

Fig. 3.—Case II. Ileum, showing the intimate connection between the diverticula and the blood-vessels.

PLATE XXIII.

Fig. 4.—Case II. Section of a diverticulum in process of formation. The atrophic mucosa is seen dipping down on either side of the blood-vessels. The muscularis is much thinned on the left and entirely absent on the right side of the diverticulum.

Fig. 5.—Case II. A later stage of development of a diverticulum. The muscularis is missing in the wall of the diverticulum. On the right it has been crowded aside by the out-pouching mucosa. Several blood-vessels with much thickened walls are seen in the wall of the diverticulum on the right.

PLATE XXIV.

Fig. 6.—Case II. Section of a mature diverticulum with greatly thickened subserosa. The left side of the diverticulum has evidently grown more rapidly than the right. A large blood-vessel is seen in the right wall of the diverticulum. The mucosa has undergone marked degeneration.

Fig. 7.—Case III. False diverticula of the sigmoid flexure. The blood-vessels are seen running over the surfaces of the diverticula.

Fig. 8.—Case IV. Appendix vermiformis, showing diverticulum, constriction and mesentery. The adhesions are not shown.

PLATE XXV.

Fig. 9.—Case IV. Section through constriction of appendix. The muscularis is wanting in this portion of the wall of the appendix.

Fig. 10.—Case IV. Section through diverticulum. The subserosa and the submucosa of the appendix are seen to merge into a single layer to make the wall of the diverticulum.

PLATE XXVI.

Fig. 11.—Case III. First stage in the formation of a diverticulum. The mucosa is dipping downward toward the dilated blood-vessel in the somewhat thinned internal coat of the muscularis.

Fig. 12.—Case III. A later stage of the same. The muscularis has become still thinner about the blood-vessel.

Fig. 13.—Case III. The mucosa has broken through the internal coat of the muscularis and has pushed the external coat ahead of it. A lateral pouching of the mucosa has taken place between the internal and external layers of the muscular tunic. The subserosa is much thickened, and in it are several blood-vessels.

Fig. 14.—Case III. The mucosa in this instance has pushed the internal muscular coat ahead of it, and with it has broken through the external one. A large blood-vessel is seen at the apex of the diverticulum.

Fig. 15.—Case III. Simultaneous rupture of both muscular coats of the intestine. Attention is called to the great number of large blood-vessels found in and about the muscularis at the site of rupture.

Fig. 16.—Case III. A diverticulum in close relation with a blood-vessel.

Fig. 17.—Case III. A diverticulum, associated with rupture of both muscular coats of the intestine, making its way along the sheath of a blood-vessel.

Addendum.—Since the completion of this paper I have examined two additional cases of false diverticula of the sigmoid and rectum (in one case with also a few diverticula in the descending colon), both being in tuberculous men—40 and 60 years old—with chronic passive congestion from cardiac and vascular disease. There were 25 diverticula in one, and 10 in the other case. The histological conditions were like those in Case III.

FIG. 1.

FIG. 2.

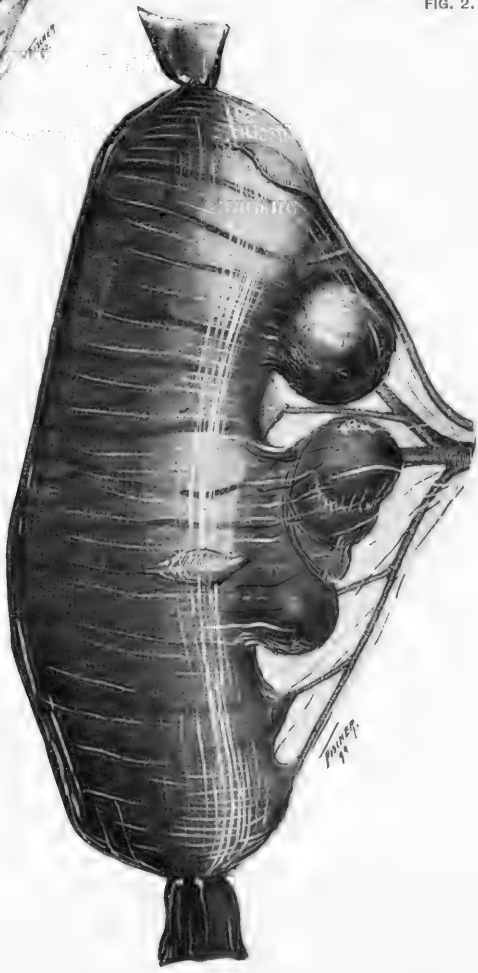

FIG. 3.

FIG. 4.

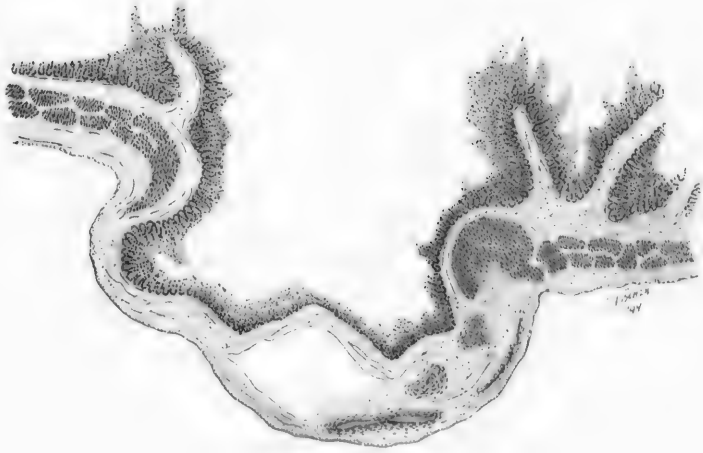

FIG. 5.

FIG. 6.

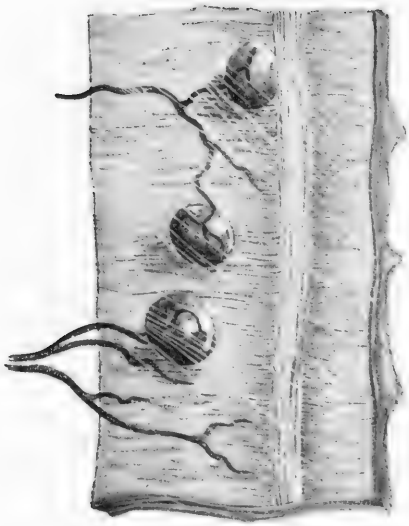

FIG. 7.

FIG. 8.

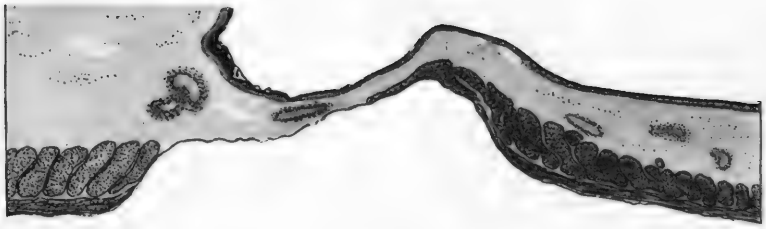

FIG. 9.

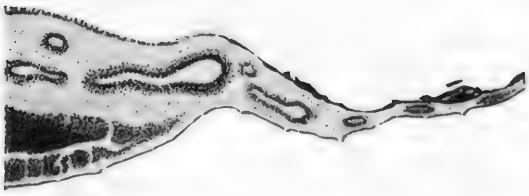

FIG. 10.

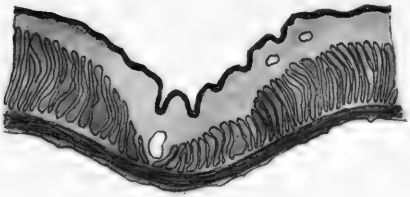

FIG. 11.

FIG. 14.

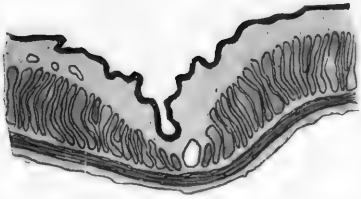

FIG. 12.

FIG. 15.

FIG. 13.

FIG. 16.

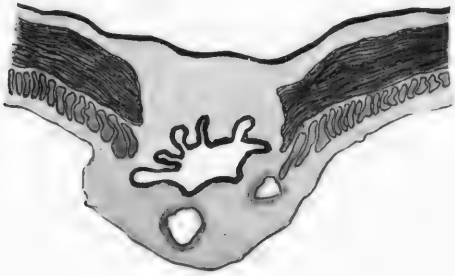

FIG. 17.

SOME THEORETICAL CONSIDERATIONS UPON THE NATURE OF AGGLUTININS, TOGETHER WITH FURTHER OBSERVATIONS UPON BACILLUS TYPHI ABDOMINALIS, BACILLUS ENTERITIDIS, BACILLUS COLI COMMUNIS, BACILLUS LACTIS AEROGENES, AND SOME OTHER BACILLI OF ALLIED CHARACTER.[1]

By HERBERT E. DURHAM, Lately Grocers' Research Scholar.

(*From the Pathological Laboratory of the University of Cambridge, England.*)

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[1] This article having been written without access to a library, I have taken the liberty of inserting in foot-notes the references to some of the authors cited in the text.—Editor.

Owing to my participation in an expedition for the study of certain tropical diseases, it is improbable that I shall be able to continue, or to render so complete as would be desirable, an account of the studies which have been made during the past five years. However, although these studies have been interrupted, the following notes may be of some interest to those working in the same field, especially since many points require further elucidation. The work upon groups of bacilli to be considered was begun in November, 1894, in connexion with the question of serum tests. These studies, together with those upon vibrios, initiated the method of testing by the clumping or agglutinating action of sera of immunised animals upon appropriate organisms.[2]

I.—AGGLUTINATING SERA.

THEIR SPECIAL ACTION UPON CERTAIN BACTERIAL RACES.

Further experience with the differential actions of sera of treated animals tends to confirm the conclusion that so far as bacteriolytic and

[2] Since many writers continue to use the name of Widal in connexion with some of these reactions, it may be pointed out that so far as priority of publication is concerned, there can be no doubt that Gruber was the first to publish the fact that human typhoid-fever patients acquire clumping power of their blood serum towards the typhoid bacillus. The whole principle of the test was established from Prof. Gruber's laboratory, and from the historical side the question of the exact period of the illness at which the reactive power is perceptible, is a mere matter of detail. Grünbaum has pointed out that some of the original cases referred to by Gruber at the Wiesbaden Congress in April, 1896, were as early as the 10th or 11th day of the fever. It may also be noted that a Fellow of the Royal Society of London was interested in the paper which I presented to the Royal Society (published in January, 1896) and which, owing to the delays in the printer's hands, was not published in full in the *Journal of Pathology and Bacteriology* until July, 1896; and he made a long abstract of this contribution which he sent to the editor of a French medical periodical. This abstract, I understand, neither was acknowledged nor was it published. Not long afterwards the application of the clumping test to the diagnosis of typhoid fever was published in France, apparently without much reference to Gruber's remarks made in April. I understand that the editor of the above-mentioned periodical was then F. Widal. Anyhow, from the point of view of priority of reference to the matter in print, if any name should be attached to the reaction it should be that of Gruber. I feel impelled to call attention to these points, since, although I personally was concerned with working out the prime foundations of the agglutination test for artificially prepared serum (and Grünbaum in the case of human typhoid fever), yet, so far as I am concerned, the inspiration was derived from my respected teacher Professor Max Gruber, as, indeed, I have already recorded in my paper in the *Journal of Pathology and Bacteriology*, 1896.

agglutinating actions are concerned, the word "specific" is inapplicable. In my previous paper[3] it was suggested that the word "*special*" would be a better one to employ. It will be remembered that Prof. Gruber and I found that within the "species" Vibrio choleræ asiaticæ the serum reactions were not uniform, in that the serum obtained by immunising with one race did not necessarily give more than a trace of reaction in vitro and none whatever in vivo when tested with another race, although it was capable of giving complete clumping and positive Pfeiffer reaction when tested upon the first race. At that time we ascribed this to differences in virulence of the races, for the serum obtained by the use of non-virulent cultures had little or no effect upon the most virulent stocks; on the other hand, the serum obtained by the use of virulent cultures affected both the non-virulent and the virulent stocks of cholera vibrios. Since then, however, I have found that certain less virulent strains of Bacillus enteritidis derived from the same source were less affected by a given serum (both in vitro and in vivo) than the more virulent strain. The matter is one which possesses considerable complexity, and we (and Pfeiffer in following us) were probably incorrect in ascribing the difference to a mere difference in virulence.

Further, in discussing the "specific" value of the test, I found that two clearly differentiable vibrios—the "Massowah," with its four flagella (not the "Massaouah" used by Bordet and others in the Institut Pasteur, which reacts as a cholera vibrio with serum, as well as in its cultural characters), and the "phosphorescent Elwers" vibrio, with its single flagellum and other special characters—were, so to speak, *serum-identical*, that is, the serum of either affected the other not only by the clumping test, but also by the Pfeiffer bacteriolytic test and the protection afforded to animals. It seemed from these observations that the serum test, both in the test-tube and in the animal, could not be considered a final criterion for the diagnosis of species; for, first, there was some want of uniformity in the action of serum tests within the "species" cholera vibrio (especially when the serum

[3] H. E. Durham, On a special action of the serum of highly immunised animals. *Journal of Pathology and Bacteriology*, 1896, iv, p, 13.

identity of V. "Berolinensis" of Rubner and V. "Versailles" of Sanarelli and V. "Iwánoff" are remembered), and secondly, there was mutual serum identity when apparently perfectly distinct "species" (such as V. "Massowah" and V. "Elwers phosphorescent") were investigated.

Since then I have made a number of agglutination tests upon members of the B. enteritidis group. So far as cultural and morphological tests are concerned, I could not certainly diagnose these races from one another, it being understood that comparative observations were made not only at a single time, but now and again in some cases over a period of years and in others of months. Temporary variations do occasionally occur, but these are not constant and, after all, are insignificant in that they do not affect main characteristics. So far as these characters go we have a well-defined group, yet, when tested by serum for agglutination, marked differentiation may be found. Thus the races "Gärtner" and "Morseele" are both strongly affected by "Gärtner" serum, whilst the races "Hatton," "morbificans bovis," "psittacosis," "Aertrycke," "Calmpthoult," "Gand," "Sirault," "hog cholera," "typhi murium," "Sheffield" (kindly sent to me by Dr. Robertson), are not very markedly affected by this serum. To some extent the race "Günther" would appear to be intermediate; still it is more markedly affected by the serum of the Hatton type. Taking the reverse view, we find that the serum of "Hatton" has comparatively slight effect upon the races "Gärtner" and "Morseele;" thus a serum which gave good reaction at 1 in 200,000 upon "Hatton," practically gave but a minimal reaction upon "Gärtner" and "Morseele" at 1:2000. It may also be mentioned that Gärtner serum, though efficient against "Gärtner," has practically no protective effect against the living Hatton bacilli; so that here within a group of bacilli, the characters of which can be more closely studied than is the case with the vibrios, the serum test does not give material aid in defining its limits. At the same time the Gärtner type has a distinct tendency to be affected by the serum derived by the use of typhoid bacilli. Here again we find differences between the typhoid bacilli, for instance the serum of the race

"Weichselbaum" has much more effect than that of my race "HS," though here it is far less than that produced upon any of the typhoid races (22) with which I have directly compared them. To put the matter in a short way, it might be said in the formula of a proportion sum that "Hatton" is to "Gärtner" as "Gärtner" is to typhoid "Weichselbaum."

Two very interesting cultures from the Pathological Laboratory of the Johns Hopkins University, "Gwyn" and Bacillus "O," for which I have to thank Prof. Flexner and Dr. Harvey Cushing respectively, have also yielded evidence that the clumping test has only a limited value in the diagnosis of species. Both of these were obtained from cases clinically resembling typhoid fever.[4] They are both distinguishable from the typhoid bacilli and also from the enteritidis group in their cultural reactions, but I hardly think that I could distinguish one from the other with certainty, so much do they resemble each other. Both of them fail to give the slightest clumping reaction with typhoid serum (potency 20,000-100,000) or with various enteritidis sera (potency 50,000-500,000); of the latter, "Gärtner," "Hatton," "Günther" and "morbificans bovis" have been tried. I prepared a "Gwyn" serum, active up to 1:20,000 upon "Gwyn," but this has not the slightest effect upon Dr. Cushing's Bacillus "O" at 1:100 dilution. Here the serum reaction confirms the notion obtained from cultural tests that these two bacilli are different from the typhoid and the enteritidis bacilli, but from their similarity it might be expected that they would shew some mutual reaction; this, however, is not the case.

One more example may be given, and it is of some value in shewing that distinctly differentiable bacilli of the colon group may simulate one another by their mutual serum reaction. Bacillus "W," a member of the B. coli communis verus race (vide, p. 371), has no power of fermenting sucrose (cane-sugar), Bacillus "G" will readily ferment this sugar; both bacilli have been under my observation for fully four years, and they have retained these characters and can be differ-

[4] Gwyn, *Bulletin of the Johns Hopkins Hospital*, 1898, ix, p. 54. Cushing, ibid., 1900, xi, p. 156.

entiated from each other by culture with ease. Now, both "W" and "G" are mutually susceptible to the clumping serum test, so much so indeed that one sample of "W" serum acted on a culture of "G" up to about 50,000 dilution, whilst upon "W" itself it fell out about 30,000.

Next it was found that apparently indistinguishable true B. coli communis races were not mutually affected by highly potent sera (none of which was less than 50,000 potency). This is a point which was foreshadowed in my earlier paper, and which, indeed, has been worked at by a number of observers with a like result. I think, however, that they have not worked with such highly potent sera, and that they thereby have saved themselves the time and patience which in some respects I have wasted. So far as my experience goes, it is rather unusual to find two otherwise indistinguishable colon bacilli from different sources, which give any mutual serum reaction in test dilutions. I have now tested a good many different cultures during the past four years and have almost always obtained completely negative results, although the sera were highly potent for their own races. In the light of the oft-repeated statements of Prof. Baumgarten, that the serum of rabbits readily agglutinates colon bacilli, I may state clearly that this is not my experience. I rarely test in lower dilutions than 1 in 200, and at this or higher dilutions of rabbit's serum (whether normal or immunised) the results are practically always negative. It is possible that his results may be partly due to the use of cultures in broth containing muscle sugar, which, when mixed with fresh broth or with serum, may give some precipitation of proteid matters and thus carry down the bacilli, giving rise to their apparent agglutination. In order to avoid such fallacies, I use agar cultures rubbed into a suspension with sterile 1 per cent NaCl solution; this is a standard method when a given parallel-sided loop and equal amounts (say about 2 mgrm.) of bacilli per 1 cc. are used. In sedimentation tubes, I find that the reading after 18-20 hours is generally the same as that taken after 48 or 72 hours, there being little or no multiplication of the bacilli.

THEORETICAL CONSIDERATIONS UPON THE CONSTITUTION OF SERUM OF IMMUNISED ANIMALS.

It should be understood that mutual reactions may be partial or complete, but in speaking of these I do not include reactions which are obtained with test sera below 1:1000. The effects produced upon bacilli by the serum of apparently normal animals in less dilute conditions can only be considered to be of a special nature, when it is *not a constant peculiarity* of the species of animal, and then to avoid fallacies dilutions not less than 1:100 must be employed. Just as we are at present unable to evaluate the position of the antitoxic action of the sera of some "normal" animals, so we cannot yet assign a position to these clumping actions of the sera of normal animals in low dilutions. Personally, I believe that both antitoxic and clumping characters in such sera owe their origin to the presence and absorption of appropriate bacillary products, but it is also possible that mutually reacting substances, *i. e.* bacillary product and serum constituent, are present, the latter of which is not necessarily identical with the true agglutinins. I may instance the precipitating effect of quillaic acid upon peptone solutions, as a case where two organic substances interact, whilst other glucoside constituents of Quillaia bark do not have this effect.

But to point my moral more aptly, an illustration may be taken from the precipitating actions of ricin and abrin upon sera. Solutions of the proteoses (albumoses) obtained from the seeds of Ricinus and Abrus (like the globulins obtainable from the seeds) form a precipitate when a drop of serum is added; the serum of normal rabbits, guinea-pigs, rats, fowls, horses and hedgehogs all produce this effect. But working with rabbits I find that the amount of precipitate obtained by adding 10 cmm. of serum to 1 cc. of a solution of Ricinus albumose is far more copious when the serum is taken from an animal which has been immunised with the Ricinus albumose than when taken from a normal or an abrin-treated animal. Moreover, the precipitation can be obtained in dilutions of the albumose solution with anti-ricin-albumose at which the normal or anti-abric serum fails to give any precipitate. Only abrin albumose precipitates both kinds of serum

(anti-ricin and normal) in like degree. I may add that the anti-ricin serum has a protective effect, whilst normal serum has none, when the serum and ricin are given in recently made mixtures.

I made some experiments with abrin, which shew that in the act of precipitation by normal serum much of the toxicity of a given solution may be removed. Thus 0.01 cc. of a given abrin solution killed 300-gramme guinea-pigs in about 60 hours, 0.02 cc. in about 48 hours, and 0.05 cc. in about 30-40 hours. To some of the same solution normal rabbit serum was added (0.05 serum to 3 cc. abrin), this was allowed to settle for a few hours and the clear supernatant fluid tested; it was then found that 0.01 cc. of the original solution failed to kill, whilst 0.05 cc. killed only after about 60-70 hours. The nature of this action is not clear and I do not propose to discuss it here. The point is that by dilutions in vitro at any rate a quantitative difference between the "coagulins" can be detected, and the protective action of the special serum is suggestive that there is a qualitative difference also.

It will be noticed that I have been comparing the bacillary agglutination process with the action of precipitating agents, more or less after the theory propounded by Kraus.[5] I do not think that bacillary agglutination is due purely to an entanglement of the bacilli in coagula formed in the free fluid. The microscopical observation of bacilli mixed with very dilute special sera is most suggestive of some alteration of the surfaces of the bacilli in the direction of increased stickiness. It may be that this surface alteration is due to a precipitation or more or less "nascent" precipitation upon the surfaces of the individual susceptible bacilli; such bacilli as are secreting more of appropriate substances will be more susceptible to the action of the serum and become more profoundly affected. It is always a striking phenomenon that all the bacilli are not equally influenced in a given dilute mixture.

In order to explain the somewhat perplexing partial and mutual reactions of agglutinating sera upon different groups or races of bacteria the following more or less graphic method may be suggested.

[5] *Wiener klin. Wochenschr.*, 1897, x, p. 736.

I suppose that a given "agglutinin" is not a single substance, but a complex one, the constituent elements of which I will designate by capital letters, whilst the bacillary components which are capable of giving rise to the formation of the agglutinins (when introduced into an animal) may be represented by corresponding small letters. Thus if we take typhoid and enteritidis sera which give some mutual reaction, they will be graphically represented thus:

Elements concerned with agglutinins	B. typhi.	B. enteritidis (Gärtner type).	B. enteritidis (Hatton type).
Serum constitution	A, B, C, D, E	C, D, E, F, G, H	E, F, G, H, J, K
Bacillary constitution	a, b, c, d, e	c, d, e, f, g, h	e, f, g, h, j, k.

When typhoid serum containing (A + B + C + D + E) is added to typhoid culture (= a + b + c + d + e) the maximum effect of clumping is produced where each substance reacts upon the other; when it is added to Gärtner bacilli (= c + d + e + f + g + h) it will only produce an effect when the substances C, D, and E are able to affect a certain proportion of the bacilli or rather their constituents c, d, and e to a sufficient degree; still greater must be the concentration of the typhoid serum to produce an effect upon the bacilli containing only the susceptible substance e.

The matter is further complicated; for instance, it may be supposed that although two typhoid races both consist of a + b + c + d + e, these substances are not present in equal quantities. Thus one typhoid race or individual bacillus may be represented by the formula 20a + 10b + 5c + 2d + 1e, and another by 1a + 2b + 5c + 10d + 20e. When tested by a third serum containing all the constituents in equal quantity (*i. e.* A = B = C = D = E) they may give identical dilution limits, but they will not do so when tested by means of their own sera. Moreover, any given race does not necessarily produce the same quantities of the different constituents at different times, and hence the variations of agglutinability, virulence, etc. Further, I suppose that the bacteriolytic, inhibitory and protective or preventive substances have a similarly complex constitution, the amount of each unit being to some degree independent of the other, though all the substances tend to be grouped together more or less

dependently. These hypothetical considerations appear to be simple and at the same time a fairly satisfactory explanation of intricate phenomena of the various actions of the sera of immunised animals.

METHOD OF PREPARING SERA FOR AGGLUTINATION TESTS.

It seems from numerous experiments that the most satisfactory method of producing clumping sera is to give considerable quantities of killed bacilli by means of intraperitoneal injections. It is possible to get moderate potency by giving sterile filtrates of cultures, as I shewed in my paper[6] in 1896, a discovery which has been variously claimed by or ascribed to Levy and Bruns or Widal, all of whom, however, described the fact about a year or so later. It is also possible to induce some power (about 1:4000 is the highest I have obtained) by giving killed cultures by the mouth; were evidence wanting that the agglutination is no "reaction of infection" this might be cited as such.

Rabbits are the most satisfactory animals to use, chiefly because a good supply of blood can be obtained from them from time to time. About one-tenth of an agar culture killed at 60°-65° C. is a good dose to begin with for a young rabbit of about 900 grammes (I prefer to begin on young animals) and this is increased gradually according to the gain in weight at intervals of a few days at first and of a few weeks later. The injections should be given on the left side of the abdomen in the inguinal region; if a blunt needle is used and the skin perforated by means of a small cautery there is no risk of wounding the intestine, for this is the region of the small intestine. The sites of the cæcum and stomach should be noted and avoided. In the guinea-pig the right side, about midway between ribs and pelvis, is the proper site for intraperitoneal injections. I have had several rabbits under treatment for periods of 2-3½ years, and they have received very many injections without causing any adhesions of the viscera.

When the dose of bacilli from 3 or 4 agar tubes has been successfully passed, it is economical and convenient to smear the surface of agar which has been allowed to set in Petri dishes. In order to smear the surface rapidly, I have made use of the turn-tables which are used for ringing microscopical specimens with cement; by removing the spring clips and putting 3 small pellets of modelling wax an excellent revolving support is made for the purpose. A quantity of culture is scraped from a gelatine or agar slope and smeared on to a broad platinum spatula

[6] *Journal of Pathology and Bacteriology*, 1896, iv, p. 13.

made of a piece of foil soldered to a wire and mounted on a holder. The dish is set spinning and the bacteria are rapidly spread.[7]

When the culture has grown the bacteria are scraped up with a loop and emulsified with saline solution and then killed by exposure to a temperature of 60°-65° C. for about half an hour.

It should be noted that broth is not a suitable substance for making the injection. I discarded it at first for reasons of economy, but the recent work upon the precipitins and coagulins, especially that of Dr. Walter Myers,[8] shews that in animals treated with peptone injections substances capable of precipitating these bodies are produced in the serum. Such a serum, when mixed with a peptone broth culture of bacilli, may cause some apparent agglutination of the bacilli by virtue of an entanglement with the precipitated proteid. A parallel effect is seen in the clumping of mica particles in a mixture of typhoid serum and filtrate of typhoid culture, as in the experiments of Kraus, or by the clumping action on indifferent blood corpuscles, as Myers has shewn.

In the treatment of the animals it is best to give injections increasing up to about four Petri-dish cultures. A series of injections of smaller quantities every day for a week, followed by an interruption and then another series of injections, is not quite so good, I think, for the preparation of highly potent sera. It appears probable that a smart reaction should follow the injection; in fact, for the highest potency the final injections should be near the maximum recoverable dose. Thus two rabbits, which had been under treatment for rather more than two years, and which had received equal doses at the same intervals for the last 12 months, were each given a considerable dose of the same material; one of the rabbits died in about 24 hours and had a clumping potency of about 1:200 at death; the other was very ill for a time but developed a potency of over 1:2,000,000. With this mode of treatment it is easy to obtain sera of a potency of 1:200,000, but it is more difficult to increase the potency up to and above 1:500,000.

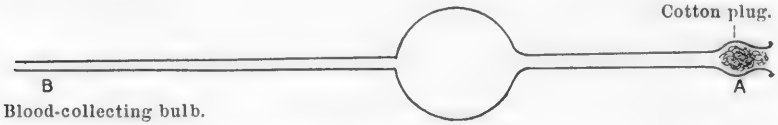
To obtain blood, the ear of the rabbit is shaved; the skin is cleansed with lysol (2 per cent) and soap and thoroughly dried with sterile cotton wool; the basal vein of the ear is compressed, and when the minute vessels are quite turgid, a small cut is made in the marginal vein by means of a sharp lancet. The blood will flow rapidly from quite a small puncture, and, indeed, will often "pump" as if from an artery. It is taken

[7] The same method is useful for separating impure cultures, the same spatula or a comb-like instrument (made by cutting a number of cuts in a piece of platinum foil) being used to smear successive plates whereby discrete colonies may be obtained.

[8] On immunity against proteids. *Lancet*, 1900, ii, p. 98.

up by means of sterile bulbs, with suction if necessary, from the pool of blood upon the skin.

Many forms of tube have been tried, but that shown in the diagram seems the most useful apparatus. The blood is then blown out into sterile test tubes, which are inclined to the maximum limit allowed by the tube in order to get a thin layer of blood clot. The tubes should not be touched until the blood has thoroughly well clotted, otherwise some blood-corpuscles will be liberated and become mixed with the serum. After an hour or so the tubes are stood up vertically. By these means some 10-15 cc. of blood and a corresponding quantity of serum may be obtained with ease from a rabbit without any operation beyond a mere prick in the vein; no pain is induced and no anæsthetic is required, the animal sitting perfectly still and shewing no signs of discomfort. The process may be repeated from day to day if desired and a considerable amount of perfectly *sterile* blood-corpuscle-free serum easily obtained.[9]



A. Mouth-piece with cotton plug.
B. Collecting capillary about 2 mm. diameter.

When it is desired to bleed the animal to death, I do so from the carotid artery, under an anæsthetic. In order to obtain a maximum quantity of serum, it is necessary to allow the blood to clot in as thin a layer as possible, and then to stand the vessel so that the clot is vertical. The most convenient means of doing so is to use flat bottles (ordinary medicine vials) into which a number of thin pieces of drawn-out glass rod are placed. These thin glass rods hold the flat thin clot so that it will not slip down when the bottle is stood up. After 24 to 48 hours the serum is pipetted off and stored in sealed glass tubes or otherwise as desired.

By this technique more, sterile, clear serum is obtained from a given amount of blood than by any other means with which I am acquainted; and I would recommend its adoption on a larger scale by those who are interested in the preparation of serum. For the preservation of the

[9] It may be added that as much as a cubic centimetre or so can often be obtained from the marginal vein of the guinea-pig's ear. This vein bleeds better than the larger veins of the ear.

serum I have never found occasion to add antiseptics, but it would appear that there is no reason against doing so, should it appear to be advisable, since for testing purposes it is rarely, if ever, necessary to use less than thousandfold dilutions, and also since, so far as clumping is concerned, the death of the bacteria to be tested is not a factor which introduces fallacies.

From numerous observations upon the course of the development of agglutinins in the blood, which I hope to publish in detail when I again have access to the records of my experiments, I am disposed to think that the effect of a given injection reaches its height (when killed bacilli are given) about 10 days or a fortnight after an injection. In following out this matter I have taken both slightly and rather highly immunised animals and removed samples of blood-serum at various periods after giving a further immunising injection. In all cases at least two samples have been taken previously to the injection so as to have ample control; samples were then taken either hourly or daily or at rather less frequent intervals and all were subjected to test with the same emulsion of bacteria. In order to gain some idea of the dilutions required for the estimation, four samples or so were tested at a number of dilutions in order to know what dilutions will be required for the final test. The tests are made by means of sedimentation in plain bulbous capillary tubes after the method devised by my friend Prof. Wright[10] of Netley. With the large number often required it is impossible to use the microscope, whilst in an afternoon several hundred sedimenting preparations can be put up. The capillary sedimentation method has the further advantage of putting all the effects before the eye at once, so that the different tubes may be put side by side and compared with one another. Moreover, for testing the potency of a serum, the microscopic method has proved very unsatisfactory as well as laborious.

After using 10 pounds of glass tubing within a period of 3 months in the form of capillaries, I came to the conclusion that it would be advisable to adopt some means of cleansing them and putting them to further use. The following method has given satisfactory results and has not been guilty of giving rise to any accidental infection to my laboratory attendant: Two sufficiently deep wide jars, one filled with solution of potassium permanganate and the other with dilute (about 10 per cent) hydrochloric acid, are required. The attendant cuts off the lower (sealed[11]) end of the capillaries with a file over the pot of permanganate,

[10] *British Med. Journal*, 1897, 1, p. 1214.

[11] It is not necessary to seal the upper end of the sedimentation capillaries.

into which each one is dropped vertically, whereby it becomes filled from below upwards with the disinfecting solution. After a day or so the tubes are removed, placed similarly in the acid and left there for a few days until the brown material is removed. They are then placed in a jar with syphon arrangement in which a constant slight flow of tap water gives them an automatic washing. When all the acid has been thoroughly removed they are shaken out and placed in a jar of distilled water (to remove lime salts) for a day or two, then they are again shaken out and allowed to dry. Lastly, they are put through the hot-air steriliser, after which they are ready for use.

For supports I keep a number of strips of sheet aluminum (about 1 inch broad and 18 inches long), each with a row of small holes (some 30-35 in number) sufficiently large to pass the capillary but not the fusiform bulbous part of the sedimentation tube. Each support is marked with a letter and each hole with a number, whereby the different specimens may be identified. When filled, the racks or supports are rested by their two ends upon a stand which formerly did service to support an incubator.

For making dilutions of small quantities and for making the mixtures of diluted serum and culture, the small porcelain pans, which are used for moist water colours, are extremely serviceable and, to my mind, more convenient than watch glasses.

Another small technical process which I have found most convenient may be mentioned. Given a sample of serum sealed in a glass tube, how is it most conveniently opened without contamination? Heat the end which it is desired to open to redness or nearly so in the flame and grasp it with the points of a pair of forceps which have been wetted with some antiseptic solution (*e. g.*, 2 per cent lysol); the end is immediately cut off and a sterile capillary tube, a number of which should be kept at hand in a sterile plugged test-tube, may be passed in and the required quantity of serum withdrawn. In this way I have removed samples of serum from the same tube over and over again without any accidental contamination; of course, after removal of sufficient serum, the point of the stock tube is again sealed.

II.—ON OTHER MEANS OF DIFFERENTIATING THE GROUPS OF BACILLI UNDER DISCUSSION.

From what has been already stated it is clear that the clumping reaction is of little value for differentiating and classifying these

bacilli in a satisfactory manner. It appears that we can only find whether the products of bacilli which are capable of giving rise to agglutinins are the same in two or more cases. Even then, although there may be the same substance or substances *qualitatively*, these may not necessarily be present *quantitatively* to the same extent. Again, by taking the same race of bacilli and its own serum, we find that the susceptibility of cultures made at different times is not necessarily the same. It seemed necessary, therefore, to enquire into other means of differentiation, but before discussing these, I propose to give a sort of classification of the types which have been studied, then to consider the ordinary characteristics which are used in laboratories, and, lastly, to give some account of media which may be useful for further work.

SUMMARY OF CLASSIFICATION.

Division I. Typhoid-like morphology: motile.

Order i. *Non-saccharid-fractors*, *i. e.*, do not ferment any saccharids.

Group A. Type, B. fæcalis alcaligenes (Petruschky).

Order ii. *Dextroso-fractors; non-lactoso-fractors:* ferment dextrose and certain other saccharids, but not lactose.

Group B. B. typhi abdominalis: no evolution of free gas bubbles; CO_2 is formed by action on dextrose.

Group C. Type, Bacillus "Gwyn"; the gas liberated under favourable conditions from dextrose not limited to CO_2.

Group D. Type, B. enteritidis: gas liberated from dextrose not limited to CO_2, even in comparatively unfavourable conditions.

Division II. Colon-like morphology: motile.

Order i. *Dextroso-non-lactoso-fractors.*

Groups E, F, and G (not to be confused with B. coli communis): differentiable by nature of growth in lactose media especially.

Order ii. *Dextroso-lactoso-non-sucroso-fractors.* Group H. B. coli communis verus: do not ferment sucrose, but cannot be subdivided by present tests except serum-reactions.

Order iii. *Dextroso-lactoso-sucroso-fractors.* Group J. B. coli communior: differ from Group H in being able to ferment sucrose.

Division III. B. lactis-aërogenes-like morphology: non-motile. *Polysaccharid-fractors.* Includes B. lactis aërogenes, B. pneumoniæ Friedländer, etc. Subdivision into groups requires further work. Ferment polysaccharids, such as starch.

DIVISION I. TYPHOID-LIKE MORPHOLOGY.

Group A. Type: *B. fæcalis alcaligenes* (Petruschky). Culture kindly sent to me by Prof. Lehmann, who obtained it from Dr. Dieudonné. Under my observation about one year.

Rate of growth typhoid-like; does not ferment nor form acid in any sugary media; not agglutinated by highly potent (*i. e.*, thousandfold dilutions) typhoid, enteritidis or coli serums, which are otherwise efficient dilutions.[12] Morphologically typhoid-like.[13]

Group B. *B. typhi abdominalis.**[14] Types: A number of different races have been utilized and some have been kept under observation for several years.

Forms abundant acid in dextrose media but no free bubbles of gas. Growth comparatively slow. Does not form any acid with lactose or sucrose. Gives clumping in high dilutions with potent typhoid serum. Sparse growth with characteristic acid formation in litmus milk whey.

[12] All the samples of human serum, typhoid or otherwise, which I have tested, clump this organism even up to hundredfold dilutions. It or some ally is probably a common inhabitant of the intestine of man, and its products are probably absorbed.

[13] B. fluorescens nonliquefaciens has been taken for this organism, to my certain knowledge. B. alcaligenes, however, gives no trace of fluorescent pigment.

[14] Serums were made from all types marked with asterisk *.

Group C. Types: *Bacillus "Gwyn"** and *Bacillus "O"* of Dr. Cushing; kindly sent me by Prof. Flexner and Dr. Cushing.

Form abundant acid in dextrose media, but free gas only when the other constituents of the medium are favourable (p. 384). No acid or gas from lactose or sucrose. Rate of growth typhoid-like. In milk whey typhoid-like. Morphologically typhoid-like. No reaction with clumping typhoid, enteritidis, colon, etc., sera.

Group D. Bacillus enteritidis group.[15] Types: Cultures of "Gärtner"* (see foot-note 14), "Günther,"* "Hatton"* (own), "Morseele," "Gand," "Sirault," "Calmpthoult," "Aertrycke," hog cholera, B. typhi murium, Psittacosis, morbificans bovis (of Basenau),* "Sheffield," "A" (own). (Unfortunately I was not able to procure cultures of Sanarelli's B. icteroides to compare with the others.) None of the above have been under observation less than one year.

Form acid and gas from dextrose. No gas or acid from lactose or sucrose. Rapidity of growth greater than typhoid; growth on gelatine in general distinguishable. Milk whey, characteristic turbidity, preliminary typhoid-like acidity (2-3 per cent), alkaline about fourth day. Serum reactions not universal within the group; three main subgroups are recognisable (see p. 356): (α) "Gärtner," "Morseele;" (β) "Hatton" and other races mentioned except (γ) B. morbificans. These subgroups run more or less into one another, thus "Günther" is more or less intermediate between (α) and (β); but there is no advantage in complicating too much.

Slight reactions with sera from certain races of typhoid, especially in the case of subgroup α. No reaction with colon, etc., sera. Morphology typhoid-like.

[15] Owing to the confusion which has been made amongst the bacteria of swine diseases, I think it well to avoid speaking of a "Hog-cholera" group.

DIVISION II.—COLON-LIKE MORPHOLOGY.

Group E. Dextrose, but not lactose, fermenter. Type: "Urethra." Given to me as a typical B. coli communis; obtained in a culture made from urethra; under observation 5 years.

Forms acid and gas from dextrose, none from lactose or sucrose. Milk whey never goes acid but becomes turbid. Morphology colon-like. Rate and appearance of growths colon-like. No reaction with any of the sera mentioned; it is capable of reaction to its "own" serum, but none sufficiently potent was prepared for trial on other bacilli on an extended scale.

Group F. Dextrose, but not lactose, fermenters. Type: "425"* (see foot-note 14). Obtained by me from fatal case of perforative peritonitis; under observation 4 years.

Growth colon-like. Morphology colon-like. Forms acid and gas from dextrose. Slow and slight formation of acid with lactose but no gas. Milk whey becomes turbid, and acid corresponds (2.5 per cent) to that of a typhoid or enteritidis culture in milk, this, however, gradually increases instead of diminishing; milk is generally loosely clotted from 10th day. No acid or gas with sucrose. No reaction with sera of other groups mentioned. Reacts well with its own serum; no other type found which reacts with its serum.

Group G. Dextrose, but not lactose, fermenters. Types: "+ A,"* "FE_3,"* and 14 others; many obtained from contaminated water near Maidstone.

Acid and gas from dextrose; acid, but no gas, in peptone-lactose solutions; no acid or gas with sucrose. Morphology and rate of growth colon-like. In milk whey, after 24-48 hours, only about 5-6 per cent acid, milk whey does not become very turbid. By serum reactions fairly well-marked group, but some races which resemble the types fail to give serum reaction; members do not react with other sera than those of the group.

Group II. *B. coli communis verus.* Types: "Escherich 1"* and "2,"* "CN_2,"* "W"* and others. I have taken this to be the standard for Bacillus coli communis, from the fact that the type species were kindly especially examined for me in Prof. Escherich's laboratory and kindly sent to me by Dr. Pfaundler. "CN_2" and "W" were types from Prof. Gruber; so far as cultural and morphological characters go I am unable to distinguish them from Escherich's type.

Acid and gas from dextrose and lactose; *none* from sucrose. Milk whey turbid, with much acid (10-15 per cent), never becomes alkaline. Mutual reactions of sera absent. No reaction with other sera tried except type "G" of the following group, which reacts with "W" mutually.

Under this heading I may mention that several observers have kindly sent me cultures. One from Prof. Flexner agrees with the types; but one "Tübingen," used as a type for class purposes in Prof. Baumgarten's laboratory and brought to me by Dr. Fawcett in 1895, is certainly no B. coli communis. In general appearance on culture media and in fermentation tests it agrees fairly with the type, but in morphology it tends to grow in threads and the flagella are quite unlike those of any other colon-like bacilli; they are quite short and very closely coiled. One peculiarity is the tendency to aggregate in flocculi in broth cultures. If this is the culture used by Baumgarten as the foundation of his statement that a high clumping power of normal rabbit's serum upon B. coli is common, it gives some explanation of his statement by insufficient controls. "Tübingen" serum, which is potent to 200,000, has no effect upon B. coli ver., nor do any of the serums tried affect "Tübingen" cultures when carefully controlled against the flocculi formation. The bacillus has been examined from time to time during 5 years, and retains its original peculiarities.

Group J. Acid and gas from dextrose, lactose and sucrose. Types: "G,"* "TK,"* "CaV,"* "CaVI"* and many others—B. coli communior.

Characters and morphology like those of group Bacillus coli communis verus except that sucrose is fermented and acid freely formed from it. Mutual serum reactions not frequently met with within the group. I am inclined to think that this is a commoner inhabitant of human fæces than members of the last group, but have not made any direct experiments. Should this prove to be the case, it might be distinguished from the Escherich type as *B. coli communior.*

Note on non-motile colon bacilli.—Besides the bacilli directly conforming to Classes H and J, there are many that I have met which differ in apparent absence of motility and also want of flagella; in general, the majority I have studied are able to ferment sucrose. It is difficult to evaluate the true worth of this, to my mind slight, morphological difference or, indeed, to be perfectly certain that there is this difference. It may be that the actual "B. coli immobilis" is not real, for the conditions necessary for the full manifestation of motility are by no means determined. It sometimes happens that after long searches in hanging drops one or two really motile individuals may be found, although the vast majority are devoid of any true locomotor power. In searching for motile power it is necessary that the medium should not contain substances capable of giving rise to directly injurious matters (such as fermentable saccharids or alcohols) by the induction of acidity. It is generally stated that colon bacilli are sluggish, and this is no doubt true for ordinary media; I once succeeded in preparing a medium (containing horse's serum and human ascitic fluid) in which the types CN_2 and G whizzed across the field of the microscope fully as rapidly as a cholera vibrio; unfortunately typhoid and enteritidis bacilli were not tried in it. The matter was not followed out, but it appears to be evident that there is a good field for research in this direction.

Then, again, there are possible fallacies in the direction of the demonstration of flagella. The flagella of colon-like bacilli are far more difficult to demonstrate than those of other allied organisms. It seems that they are more readily shed and broken off than in the case of typhoid, enteritidis, etc., bacilli, especially when staining methods necessitating much washing and many transferences from one fluid to others are employed. Notwithstanding this defect, van Ermengem's silver method is, on the whole, to be preferred. To illustrate the fracture and disappearance of flagella by this method, I once made a batch of coverslip smears from an emulsion (race CN_2) and stained some by van Ermengem and others by a method resembling that of Pitfield, but in

which saturated stanneous chloride was used in place of alum. (This gives very intense staining with the single fluid, but is apt to give very much precipitate, although I have sometimes obtained quite clear specimens. Formula: sat. sol. $SnCl_2$, 1 pt., 10-15 per cent tannin 1 pt., sat. alcoholic methyl violet few drops; warm; put in the coverslips and allow to cool; wash in distilled water. I may note here that in methods like this all precipitate and indeed all stain may be removed by treatment with warm tannin solution and the specimen restained.) In the van Ermengem specimen the flagella were short and many of them broken off, whilst in the tin-method specimen the flagella were mostly retained and fully twice the length of those in the van Ermengem one. It is erroneous to state that the flagella of B. coli are shorter than those of typhoid bacilli; when complete they are fully as long. By repeated observations of apparently non-motile cultures I have occasionally succeeded in finding isolated flagella-bearing individuals. I am inclined to think that the absence of conditions favourable for the development and demonstration of flagella may lead to a false conclusion as to motility and the presence of flagella.

DIVISION III.—B. LACTIS-AËROGENES-LIKE MORPHOLOGY.

Division of B. lactis aërogenes.—Fermenters of polysaccharids, such as starch. Type: B. lactis aërogenes, "Kosseck" obtained from Prof. Escherich's laboratory, "Brad 1,"* "Brad 2,"* "Aërogenes"* from typhoid stools and many others.

Besides possessing the power of fermenting dextrose, lactose and sucrose, these groups can ferment also substances like starch and inulin. Some have very strong reducing power, and also great power of surviving and overcoming considerable amounts of acid which they have produced. They are non-motile, without flagella, and plumper than B. coli. They do not form chains or threads and are not often seen even in pairs. B. mucosus capsulatus, Friedländer's pneumobacillus, and also the "Schweinepest" bacillus used by Voges and Proskauer, are closely allied to this division. All of those which I have tested give the pink-red reaction of Voges and Proskauer,[16] and this possibly may be regarded as a group colour-reaction; it is *not* given by any of the members of the other divisions which have been classified above. The bacilli are grown in a peptone-sugar-salts solu-

[16] *Zeitschr. f. Hygiene*, 1898, xxviii, p. 30.

tion for a day or two and then about 1 cc. of strong caustic potash is added; an eosin-like colour appears after a while near the surface and lasts several days; the pink colour may appear as early as an hour after the caustic is added; Voges and Proskauer only describe its appearance after a day or so. The red colour may be quite intense and slowly fades away generally after several days.

There is no mutual serum reaction between the members tried nor with the other sera (typhoid, etc.) which have been prepared.

This group ("lactis aërogenes" of Escherich) is divisible into subgroups by the aid of fermentation tests, thus the "Kosseck," which, as coming from Escherich, may be regarded as "B. lactis aërogenes verus," is able to produce acid and gas from peptone-starch but not from peptone-inulin media. Other types will ferment both starch and inulin; others again inulin only. The power of fermenting starch no doubt has led to the description of the frothy culture on potato.

On gelatine plates the colonies of this group are bulky, white and moist, without the tendency to spread which characterises the colon-like organisms; consequently they are circular in outline. In slope cultures there may be a tendency for the growth to slide down when the surface is vertical.

BACILLI NOT TO BE CONFUSED WITH THE FOREGOING GROUPS.

One not infrequently meets with organisms which simulate the thin, spreading, irregular colonies with brown translucency of the colon and of the somewhat colon-like groups in gelatine plates. These, so far as I have observed them, are distinguishable either by morphological characters, such as chain and thread formation, or in cultures. Some of them are incapable of producing acid or gas with the three sugars, whilst others have some power in this direction. Some observers have gone so far as to include bacilli which are capable of liquefying gelatine under the colon group. This seems to me to be a childish disregard for one of the prime criteria of Bacillus coli communis. I have met with organisms which have the power of slowly liquefying gelatine media and which at first on gelatine plates had some resemblance both to B. coli communis and to B. lactis aërogenes. Any cultures about which there is some doubt should be kept on sugar-free gelatine media for 8-10 weeks, by

which time liquefaction will probably set in if it occurs at all. In general, I find these types do not produce acid in milk whey nor do they produce acid from lactose in broth. One culture of such an organism was kindly sent me by Dr. Mervyn Gordon; in his description of it in his paper,[17] he states that it is agglutinated by typhoid serum. I have tried cultures of it with typhoid sera of potency 1:20,000-1:50,000 without the slightest trace of positive reaction at 1:100 and I have also prepared a serum with it of potency 1:200,000, which has not the slightest action upon several typhoid or enteritidis races even at 1:100 dilution. It is to be surmised that the technique adopted by him was faulty, as indeed is suggested by his description of his experiment.

REVIEW OF SOME TESTS ORDINARILY APPLIED.

Morphology.—Speaking generally, morphological characters are not of much value for subdivision of these bacteria. Among the organisms with typhoid-like morphology, I find that some individuals, as B. morbificans bovis, are not described as forming threads in cultures; in my hands this bacillus readily forms threads in broth and in agar.

The characteristic staining of the enteritidis group when grown at room temperature, especially upon gelatine, I have not met with in any other group or division. There is perhaps a tendency for the appearance of stained middle and unstained ends with the Bacilli of Gwyn and of Cushing, though I have not obtained very characteristic preparations such as are obtained with the enteritidis group.

It may be noted that flagella are of no value as a classifying test; it is not possible to differentiate between groups A, B, C and D by their means. The numbers of flagella given by many observers are not reliable, and are usually too low. The number of flagella will also not differentiate between the different groups of the more colon-like organisms. They are, therefore, of no value for ultimate classification.

Cultural Tests. Normal Agar and Gelatine Media.—The former does not afford any particular aid to differential diagnosis, although differences, such as the free growth of B. aërogenes, should be noted. The latter may give some idea of probabilities between the main

[17] *Journal of Pathology and Bacteriology*, 1897, iv, p. 446.

divisions. By continued transference upon gelatine for many months or several years, as in keeping the collection growing, I find that the tendency to spread and form thin layers is frequently lost by the typhoid and sometimes by the more colon-like races; in general, the enteritidis has not much tendency to spread, although each of the races I have had does so from time to time. It should be noted that the gelatine for a slope culture must always be melted and sloped within a few hours or minutes of the time of sowing; gelatine which has been sloped for days becomes dry on the surface and this tends to prevent the spread of all but the most hardy races. Gelatine does not avail for discrimination between many of the groups.

Broth and "Peptones."—The normal broth I use is made from stale meat (beef or ox-heart) which has been hung for 3 or 4 days, infused for about 24 hours, strained, and heated after addition of 1 per cent Witte's "peptone" and 0.5 per cent pure NaCl. This is the basis of the gelatine and agar media. The neutralisation is done by caustic soda until a decided pink tinge is obtained with *rosolic acid* either on a porcelain plate or by cautiously dropping the dilute almost colourless indicator on the surface of a sample. The end reaction is practically at litmus neutral point. Phenolphthalein appears to me to be a clumsy indicator and the growths of the bacilli which I am dealing with do not appear to thrive nearly so well in media in the preparation of which this indicator is used. I have received several cultures from the Johns Hopkins laboratory and these, as well as others which have been sent to me from elsewhere, have given markedly more luxuriant growths in my hands than the original cultures from which they were planted; whether this is due to the reaction or the general constitution of the media is more than I can say.

In broth cultures of the enteritidis group, I have always found general turbidity of the medium and that a pellicular growth formed on the surface. In such broth no pellicle is formed by typhoid, "Gwyn" or colon groups. No pellicle occurs when simple "peptone" (Witte) and salt solutions (with or without sugars) are planted with any of the groups.

By digesting meat with pancreatic ferment (the "zymine" of

Burroughs and Wellcome was used), the resulting broth being made faintly alkaline (as above) to rosolic acid, most luxuriant growths occur with all the groups; and (except B. lactis aërogenes, which was not tried) abundant pellicle formation occurred. In this medium I find that typhoid races grow more freely than colon races do in "normal" broth. On the other hand, broth (and gelatine media made from it) made by digesting meat with pig's stomach and acid (L. Martin) is a very unfavourable medium for all these organisms; in general (including B. lactis aërogenes) they grow in floccular masses and leave the broth almost clear.

When broths for sugar tests—either the normal or pancreatic varieties—were made, they were first inoculated with B. lactis aërogenes and then with B. coli communis until no trace of acid or of gas was generated.

Milk.—Milk is not a satisfactory differentiator. It fails to distinguish groups A, B, C, D and E from one another, although these are so widely different. It also fails with H and J, early clotting occurring in both cases. Even when litmus is added it does not become much better, since owing to its initial alkalinity it does not shew differences markedly between A, B and E. Owing to alkali formation (after a preliminary diminution in some cases) a certain amount of clearing may occur (due to saponification?). This is especially the case with the enteritidis group, where it may occur after about 2 or 3 weeks; it may also happen with the typhoid group after a few weeks, as I have not infrequently seen, but it has generally occurred in tubes which have been left untouched in the incubator. Since the alkali formation is dependent upon growth in contact with the air, slight jarrings by removal may interfere with the phenomenon (compare the effect of movement in the preparation of diphtheria toxins).

If dextrose (1 or 2 per cent) is added, the milk becomes highly acid (*e. g.*, 10-15 per cent to normal alkali) with typhoid and enteritidis, but is not clotted. The clotting caused by B. coli is commonly ascribed to the acidity, but in this case it must be due to some special acid. According to Blachstein,[18] B. typhi produce a lævo-

[18] *Arch. d. sciences biol.*, St. Petersb., 1892, i, pp. 199 and 299.

rotatory lactic acid from dextrose whilst B. coli gives a dextrorotatory one.

Potato.—Potatoes are too uncertain in their constitution (especially their acidity) to be reliable as a test. Moreover, it is impossible to distinguish by cultures on potato such widely different groups as E, F, G, H and J. Potato is therefore useless for purposes of discrimination.

Test Media of Capaldi and Proskauer.—The two media ((1), 2 per cent Witte peptone, and 0.1 per cent mannite, (2) asparagin medium with salts and glucose or mannite) recommended by Capaldi and Proskauer,[19] are also of slight differential value. I find the peptone medium remains acid with groups B and C (typhoid and Gwyn) for several days, whilst it fails to differentiate between D, E, F, G, H and J. The other medium does only what is claimed of it for the typhoid group. After 24 hours' incubation, according to the vigour of growth, it soon becomes acid. I do not think that it is to be accorded any very high position as a test.

Indol.—The presence or absence of indol in cultures of these groups of bacilli is not of value for differential methods. Thus group G forms but a trace of indol, much like the amounts formed by typhoid and enteritidis. The most abundant indol production I have met is with Bacillus "425" (group F). There does not appear to be any relation between the amounts formed by the sucrose-fermenters and the non-sucrose-fermenters, nor between the lactose- and the non-lactose-fermenters, if we compare type "G" with "CN_2," or "Urethra" with a true B. coli communis of Escherich, in which the quantities of indol are about the same.

Semi-gelatinised Media.—These I have not tried. Since the designers have merely claimed that their action is due to the diffusibility of more highly motile organisms, it is not to be expected that groups A, B, C and D would be differentiable by this means.

Litmus Milk-whey.

Up to this point I have acted the part of a destructive critic, but I cannot speak too highly of this medium. It is largely upon it as a

[19] *Zeitschr. f. Hyg.*, 1896, xxiii, p. 472.

basis that the present grouping or classification of these organisms has been made. In fact, according to my experience, more can be learnt from the course and appearance of cultures in this medium (titrated for acid if necessary with one-tenth normal NaOH) than *with any other single medium*. It is easy to prepare, but the method originally described by Petruschky,[20] who devised it, is not to be recommended in that he advocates the use of mineral acid, whereby some change of the lactose into dextrose and galactose might occur.

The following method gives admirable and apparently very constant results: Fresh milk (free from antiseptic adulterations) is slightly warmed and clotted by means of essence of rennet. The whey is strained off and the clot hung up to drain in a piece of muslin. The whey, which is somewhat turbid and yellow, is then cautiously neutralised, neutral litmus solution being used as an indicator, with 4 per cent citric acid solution. When it gives a good neutral violet colour with the litmus, it is heated upon a water-bath at 100° C. for half an hour or so; thereby nearly the whole of the proteid is coagulated. It is then filtered clear and neutral litmus is added to a convenient colour for titrations or rougher observation. Lastly, it is measured off by a burette into quantities of 10 cc. per tube or else sterilised at 100° C. in bulk. If difficulty is found in obtaining it perfectly clear it may be sterilised in bulk and allowed to settle for a few days or it may be passed through a Berkefeld filter; this, however, is rarely necessary to the practised laboratory attendant. *It must never be heated above 100° C.*

The value of this medium is partly due to the almost complete absence of proteid, to the presence of lactose and to the presence of a small quantity, perhaps about 0.1 per cent, of a sugar of the nature of dextrose or galactose (vide infra). Theobald Smith [21] has recently called attention to the presence of the latter constituent in milk, and I may say that I came to the conclusion some while back that there was some other saccharid in milk besides lactose; the exact determination must be left to the chemist. The contained salts (which by the

[20] *Centralbl. f. Bakter.*, 1889, vi, p. 657.

[21] *Journal of the Boston Society of Medical Sciences*, 1898, ii. p. 236.

way do not interfere with the colour or titration accuracy of the fluid) may also be of value in determining the results.

Group A. B. fæcalis alcaligenes clouds the whey and produces alkali without previous formation of acid, and is thereby at once distinguished from members of groups B, C and D.

Groups B and C are much alike; after 24 to 48 hours at 37° C. the medium is hardly perceptibly turbid but has become distinctly acid (= about 3 per cent normal soda); the reaction after this remains about the same.

Group D begins with an acidity of from 2.5-4 per cent, the medium being made quite turbid. About the 4th day, at 37° C., it commences to become alkaline and may soon reach about 16 per cent normal H_2SO_4.

Group E, type "urethra," never forms acid in whey. It is distinguished by growth in glucose media from A.

Why this organism does not produce any acid is not clear; possibly the second sugar is not dextrose or possibly alkali is formed too rapidly for the manifestation of acid.

Group F produces an enteritidis-like culture at first, but the acidity continues and increases.

Group G produces 5-6 per cent of acid; the culture is only slightly turbid in 24-48 hours and never becomes alkaline.

Groups H and J give at least 10-15 per cent of acid in 24-48 hours; the fluid is turbid and never becomes alkaline.

Group B.lactis aërogenes makes acidity and turbidity; in vigorous growths the colour of the litmus is completely reduced except a thin film on the surface; upon shaking and aëration the red colour appears.

These short notes will give some idea of the value of the medium. No artificial medium that I have tried has given such good results.

FERMENTATION TESTS.

Methods.—The apparatus for determining the production of gas bubbles which I described some time ago [22] has enabled me to carry

[22] *British Med. Journal*, 1898, i, p. 1387.

out a very considerable number of fermentation tests. The apparatus consists merely of a small test-tube inverted within an ordinary one; the medium is poured in and the process of discontinuous sterilisation causes the inverted tube to become completely filled. It is preferable to use fairly recently sterilised media, for when kept for a few weeks air becomes dissolved and may appear as a bubble in the collecting tube if acid is produced by the bacillus under observation, giving a false impression of gas production. In all these tests it is advisable to do considerable batches at a time, both of known and unknown organisms, in order to be sure that the medium reacts towards the known in the way it should. It is also not a bad plan to do a double set and leave one undisturbed in the incubator whilst the other is examined day by day. The routine of examining after 24 and 48 hours, and then on the 7th, 14th and 21st days, seems to be ample.

When only CO_2 is formed by destruction of a sugar (as in the case of glucose with typhoid) no gas collects in the collecting tube as a rule, but when the evolution is caught in full vigour some may sometimes be seen in the inverted tube. When both H and CO_2 are given off, some CO_2 collects in the tube; this may be absorbed by caustic solution if desired, but I find that a sojourn of about a week in the incubator has a precisely similar result in that all the CO_2 has disappeared. I have not made any estimations of the relative quantities of H and CO_2 in the fermentation tubes, since these would be of no value whatever. To obtain ratios of value, the *total gas* formed must be collected and extracted by vacuum over mercury before estimation. The fad of recording the ratios CO_2 to H, obtained with ordinary open fermentation tubes, appears to me to be a sheer waste of time and trouble.

FACTORS TO BE CONSIDERED IN APPRECIATING FERMENTATION TESTS.

Several factors have to be considered in appreciating the results of growths in media containing sugars, fermentable alcohols, etc.

It is of prime importance to know that the sugar employed shall not have been changed in its constitution during the process of making the medium. It is of no less importance to ensure that other con-

stituents of the medium are unable to give rise to fermentations. To ensure these points:

(*a*) No sugar-containing test medium should be exposed to the action of free acids (especially mineral acids) or caustic alkali.

(*b*) No sugar-containing test medium should be exposed to temperatures above 100° C.; indeed, the lower the sterilising temperature used the better.

(*c*) No sugar-containing test medium should be heated at all, except it be in a *neutral* condition, some such neutral point as that of litmus being used.

(*d*) No sugar-containing test medium should become yellower or browner than the stock solution to which the sugar has been added for the preparation of the medium; the change of colour shews that some change has taken place. (The medium called dextrose-broth, or dextrose-gelatine, seen in most laboratories is manifestly browner or darker than the plain media. This only means that the medium is not truly a dextrose-medium; the addition of dextrose to a medium should produce no change of colour if it is to be used for test purposes.)

(*e*) The stock solution to which the test sugar is to be added must be tried with appropriate cultures to prove the absence of fermentable matter. It should be ascertained that no acid, as well as no gas, is produced. This is especially necessary where meat-broths are used. It may be necessary to plant broth several times with enteritidis, colon, or aërogenes cultures before it is finally made up with sugar.

(*f*) Eggs should not be used for clearing media destined for fermentation tests, the ovomucoid and other constituents being capable of being split up.

(*g*) When tests are made upon sugary or fermentable media, they should be efficiently controlled by means of cultures of known bacilli in the same batch of medium.

(*h*) It is well to see that tested cultures are really pure and also that any given test gives the same result on repetition as it did on first trial.

Especially in the trial of media of unknown constitution with bacilli

of known proclivities, it is necessary to pay heed to the following facts, which are also not without importance for general purposes:

1. *The result depends on the nature of the sugar, etc., employed.* By means of the groups of bacilli we are dealing with it is possible to determine that certain sugars may be present or that they are absent. But they will not shew differences between certain sugars. Thus dextrose, lævulose, mannose, arabinose (a pentose), galactose and maltose (a disaccharid) give much and apparently fairly equivalent quantities of acid with races which are able to decompose dextrose (*e. g.*, typhoid) or acid and gas with races (enteritidis and coli) which are able to ferment dextrose. This statement is founded on titrations of 1 per cent solutions containing 1 per cent Witte's peptone; very exact titrations with equimolecular solutions have not been tried.

Mannite and dextrin also react like dextrose, in that those bacilli which will make acid or gas with one of the former will do so with the other; less acid appears to be formed from the dextrin, which was carefully tested with Fehling's solution to see that it was sugar-free.

The groups under discussion fail in a general way to distinguish between these different substances; group A forms no acid from any of them. This being the case, it did not appear to me to be advisable to follow the suggestion kindly given me by Dr. Ruhemann to compare samples of these sugars of different configuration or rotatory power. It may be that the finer discrimination or greater differentiation of these or other bacilli may permit of less coarse work and greater discrimination.

With dextrose, lactose and sucrose, and starch, inulin and glycerin in conjunction with the use of milk whey, much may be done in classifying the bacilli which belong to these groups; the morphological characters, the rate of growth in normal media, the non-liquefaction of gelatine and appearances thereon and the decolorisation by Gram's method not being omitted.

2. *The result depends upon the amount of the sugar present and the power of overcoming initial acid formation.* Capaldi and Proskauer[23] have shewn that some bacillary cultures tend to remain acid

[23] *Zeitschr. f. Hyg.*, 1896, xxiii, p. 452.

when small quantities of glucose or mannite are present. Thus typhoid cultures in 1 or 2 per cent peptone with 0.1 per cent dextrose, and, I might add, with maltose, etc., tend to remain acid; eventually, however, this bacillus is capable of overcoming the acid and the culture then becomes neutral and eventually alkaline. I find, similarly, with 0.1 per cent lactose and a Bacillus coli communis, or 0.1 per cent sucrose and a Bacillus coli communior, after 24 hours' incubation there is no acidity, the acid formed being rapidly neutralised. On the other hand, 1 per cent of either sugar with the appropriate bacillus yields a permanently acid culture. It is evident, therefore, that the amount of the fermentable substance present must be duly considered.

3. *The result depends upon the favourableness of the other constituents of the medium to which the fermentable substance is added.* It is not merely necessary to have a given fermentable substance in a medium, the remaining constituents must be sufficiently favourable to the growth of the bacillus to be tested.

For most of the groups a plain solution of Witte's peptone 1 per cent and sugar 1 per cent is sufficiently rich in nutritive material for gas production, given a bacillus which has the power of fermenting the sugar. In the case of organisms which are not able to flourish well in such simple media no gas may collect, although the sugar is fermentable. I have repeatedly tested Bacillus "Gwyn" at different times (and also Bacillus "O" of Cushing less often) for gas formation in 1 per cent Witte's peptone and 1 per cent dextrose. It appears from these oft-repeated tests that, although the medium is acidified, no gas bubbles collect; in fact, so far as appearances go, the cultures might well be of typhoid bacilli. Very different, however, is the result if 1 per cent of dextrose is added to sugar-free "normal" or "pancreatised" broth; here in both cases, besides the abundant acid, much gas is formed.

The action of B. enteritidis (a hardy race) upon glycerin may be in the same category. On repeated observation I note that only acid and no gas is produced by growth in 1 per cent Witte's peptone and 1 per cent glycerin. On the other hand, all the groups from E on-

ward have the power of producing gas from this simple medium. I find, however, that Voges and Proskauer,[24] in their paper on the bacteria of hæmorrhagic septicæmia, say that the hog cholera bacillus ferments glycerin added to their peptone and salt solution. While I have not observed gas formation with this bacillus growing in simple peptone-salt solutions containing glycerin, it may be that the more nutritive meat broth may enable the bacillus to produce gas from glycerin. This is a point which I was about to determine when I left England on the present expedition.

Further experiments of a like nature were contemplated to determine the simplest media compatible with gas formation by more and less hardy groups. Some preliminary experiments were made with proteid-free media, such as asparagin solutions and sodium-urate solutions. The latter give very pretty results if saturated solutions are used with sugars, for where acid is engendered the uric acid comes down in beautiful acicular crystals.

4. *Is the sugar altered by growth of a bacillus which does not produce acid or gas?* I have made experiments, for instance, by growing B. fæcalis alcaligenes in glucose, and, after awhile, planting the medium with B. typhi; abundant acid is then produced. Or after growing B. enteritidis in lactose media, subsequent planting with a B. coli gives rise to acid and gas. These and other experiments shew that even if the sugar is altered, the resulting bodies are fermentable by those bacilli which are ordinarily capable of so doing.

Preliminary Search for other Sugars and Fermentable Substances.

It seemed possible that there might be many substances of carbohydrate nature which would serve for further differentiation of these and other bacillary groups. After consulting Tollens' "Handbuch der Kohlehydrate" and Landolt's "Das optische Drehungsvermögen," the most useful mode of attacking the question appeared to be an empirical haphazard one.

Various substances, such as fruits, seeds, etc., were collected and extracts of these were made in the cold state when starch, inulin, etc.,

[24] *Zeitschr. f. Hyg.*, 1898, xxviii, p. 20.

were present or suspected. The extract was then tested for its power of reducing Fehling's fluid both before and after hydrolysing with a trace of dilute sulphuric acid. Plain neutral litmus solutions [25] were made according to the indications of the amount of sugar present. These were put into my fermentation tubes and tried with beer yeast and two members of each of the above groups for acid and gas formation. Great care was taken that no extract was heated except in a litmus-neutral condition. Again, other media were first planted with yeast and thoroughly freed from any substances which this could destroy before testing with the bacilli.

Among the sources of sugars which have been tried the following may be mentioned: Truffles, yeast, potato juice (starch-free), juice of Jerusalem artichoke, milk of ripe cocoanut, acorns, madder root, mangold-wurzel, carrots, fruit of Cratægus oxyacantha, C. pyracantha, C. punctata, Rosa, medlar, Solanum nigrum, S. dulcamara, tomato, egg plant (brinjal), Physalis, Cytisus laburnum seeds, Digitalis leaves, privet berries, holly berries, mistletoe berries, ivy berries, fir cones, Abrus seeds, bananas and many other sources. In several instances the hydrolysed, as well as plain, extracts were tested. A few glucosides (*e. g.*, Quillaia) have also been tried.

It was intended to go more fully into those substances which appeared to promise results, and to purify the fermentable substance. This I have as yet been unable to do. After purification it was proposed to retest in media made of favourable constituents.

I shall not go fully into the results obtained up to the present. The work upon this line was commenced early last year (1899), but was much interrupted by other calls upon my time. But anyhow, this mode appears to open up an enormous field for research both by the bacteriologist and the chemist. A few points of interest may be noted. In the first place the plain extract from the fruits, seeds, etc., may not have sufficient pabulum for fermentations by the bacilli; thus a plain extract of hawthorn gave no gas with group C and one of medlar gave the same result with groups C and D, although acid

[25] Much credit is due to my laboratory attendant, Mr. W. Mitchell, for the preparation of these media; he managed to produce beautifully clear media from the most unpromising materials.

was formed. When peptone or broth (sugar-free) was added to the medium free gas evolution took place when the same bacilli were cultivated in it. In fact, all through the work with many of the extracts the difference between groups C (Gwyn and Cushing) and D (enteritidis) was exemplified time after time, the former giving cultures like those of typhoid. A few substances deserve particular mention:—

Yeast-broth.—The first lot tried was made from baker's yeast on Spronck's formula.[26] Much CO_2 was formed by all the group types: in only Cushing, Gwyn and aërogenes, however, did permanent (non-absorbable to NaOH) gas result. This appeared to promise well as a differential test between C and all the other groups except aërogenes. However, on repetition, I was unable to obtain exactly the same result, although the first test gave a most striking and apparently unequivocal result. This requires further work.

Cocoanut milk.—This was obtained from the ripe cocoanuts we get in England. According to Tollens, it should contain the disaccharid, cane-sugar or sucrose. On testing with Fehling it gives no reduction until it has been hydrolysed. If this non-reducing sugar were really sucrose, it should not be attacked by any of the groups but "J" and B. lactis aërogenes. As a matter of fact, however, even the typhoid bacillus produces much acid and all the other cultures much gas as well. It is clear, therefore, that the sugar is different from the ordinary sucrose; it reacts towards these bacilli like dextrose or more accurately the disaccharid maltose.

Jerusalem artichoke.—The filtered juice contains a similar sugar in that it gives no reduction of Fehling till inverted. With the cultures it acts like the cocoanut sugar. The same is true of tomato, mangold-wurzel, Solanum dulcamara, etc.

Glucosamin, prepared from decalcified crab-shells by sulphuric acid, reacts like dextrose with the bacilli. With group C the addition of something more than plain peptone is necessary for gas production. I need hardly add that this substance is not attacked by pure cultures of yeast.

[26] *Annales de l'Institut Pasteur*, 1898, xii, p. 702.

Potato juice, starch-free, is not without interest. It contains a substance capable of reducing Fehling, but it is attacked only by B. lactis aërogenes.

Acorns contain a body which is fermented by the sucrose-fermenters, but is not attacked by the other groups. This substance is destroyed by English beer yeast so that no fermentation occurs with the sucrose-fermenters after the action of the yeast.

Gums, such as arabic, tragacanth and agar-agar, are not fermented by any of these groups until hydrolysed.

It would be idle to pretend to do more at the present time than to call attention to the wide field for research which is thus opened. Much of the information upon sugars in books on chemistry, especially in regard to the fermentations by yeasts, seems most unsatisfactory. It does not appear at all clear (vide Tollens) that in general pure yeast cultures were used nor indeed what species of yeast was used.

If this contribution should stimulate further and more accurate work, both bacteriological and chemical, in this interesting field, it will indeed be more than this incomplete account of my preliminary work on the subject can claim to merit.

Pará, Brazil, August, 1900.

REPORT OF A LABORATORY EPIZOOTIC AMONG GUINEA-PIGS, ASSOCIATED WITH GASEOUS EMPHYSEMA OF THE LIVER, SPLEEN AND KIDNEYS, DUE TO BACILLUS MUCOSUS CAPSULATUS.

By R. G. PERKINS.

(*From the Pathological Laboratory of The Lakeside Hospital, Cleveland.*)

During the summer of 1899, an epizootic broke out among the stock guinea-pigs belonging to the laboratory, in the course of which twenty-five animals were affected. Careful watch was kept over the stock in order that such animals as showed symptoms of illness might be at once isolated; in this manner the course and duration of the infectious process in the various cases could be carefully watched and noted.

The symptoms were similar in all cases; the animals ceased to eat, their hair became much ruffled, and their condition grew rapidly worse, culminating in coma, which existed for some time before death. During the coma there were intervals of muscular twitching, which in one or two of the animals were sufficiently marked to be called convulsions. In the animals autopsied as soon as respiration and cardiac impulse had ceased, so far as external examination could determine, the heart was found to be still beating very slowly. The heart as a whole, the separate divisions, and even strips of the ventricular walls, responded readily to stimuli for fifteen minutes or more after removal from the body, though no precautions were taken to prevent drying.

The duration of illness in the fatal cases, from the first symptoms noted to the time of death, varied between 12 and 48 hours, but by far the greater proportion died in from 18 to 24 hours after the onset of the disease. Two of the infected animals recovered after an illness of seven or eight days. In these cases the condition of coma was not reached, but it was over a week before the animals began to eat, and between two and three weeks before they recovered their normal weight and health.

Careful autopsies were made in all the fatal cases from 15 minutes to 24 hours after death. The bodies were kept in the cold chamber until autopsied, at a temperature well below the freezing point.

Summary of autopsy protocols.—None of the bodies showed any swelling after death, nor could emphysema be detected by external examination. Further examination of the skin and subcutaneous tissues failed to demonstrate any emphysematous areas. The glands of the groin and axilla were slightly swollen.

Thorax.—The pleural cavities showed no adhesions and no excess of pleural fluid. The lungs were normal except in one instance, where there was a lax consolidation of the posterior part of the right lower lobe. The pericardium and heart were uniformly negative.

Abdomen.—In 15 cases, or 65% of the animals autopsied, there was a well-marked peritonitis, chiefly of the sero-purulent type, though a few flakes of fibrin were usually to be found in the dependent parts.

The liver in every case showed marked congestion and cloudy swelling. In 14 animals, or 60%, there was a general gaseous emphysema of this organ which in 4 instances was so extensive as to resemble closely the "Schaumleber" described in cases of invasion by B. aërogenes capsulatus. No areas of degeneration were noticed macroscopically.

In 6 of the animals there was associated with this hepatic emphysema a like condition of the spleen. This organ was markedly congested in all cases. In 8, or 39% of all, there was gaseous emphysema like that described in the liver. In 2 of these cases, the emphysema was so extensive that the splenic capsule was distended until the organ was cylindrical in contour, resembling a small sausage.

The kidneys and adrenals showed no macroscopic changes other than well-marked congestion and cloudy swelling.

The gastro-intestinal tract contained much gas, but no lesions could be demonstrated.

Bacteriological examination.—Coverslip preparations were made at each autopsy from the heart's blood, the liver and the spleen, and from the peritoneum in cases with inflammatory exudate. The coverslips were stained in various ways, both with the usual aniline dyes and with Welch's capsule stain. In all instances of peritonitis, large numbers of organisms were found in the exudate, and in most cases the blood and organs showed a small number of bacteria of the same type. The organism uniformly seen in the stained smears was a short bacillus, with rounded ends, 0.5 μ to 3 μ in length, and about 0.5 μ in diameter. These occurred most frequently in pairs. Examination by the hanging-drop method showed absence of motility. Capsules were readily demonstrated by Welch's method. The organism stained easily with the aniline dyes, and decolorized rapidly when treated according to Gram.

There was no pleomorphism observed in stained specimens from the organs and exudates.

Cultures were made at each autopsy from the heart's blood, the liver, the spleen, and from the peritoneum when an exudate was present. The usual precautions were taken to prevent contamination, the surface of the organs being seared with a hot knife before the insertion of the platinum loop. Glycerine-agar slants were used, and from these, when the organism had grown out, transplantations were made into glucose-agar, glucose-bouillon, gelatin, milk, and on potato. With two exceptions, each of these being from a peritoneal exudate, the original tubes showed pure cultures of the organism to be described. In the two mixed cultures there were a few colonies of Staphylococcus aureus in addition to the characteristic bacillus. The tubes inoculated from the peritoneum showed a profuse growth, while in those inoculated from the blood and organs the colonies were fewer and often separate. The superficial colonies were round, soft, grayish-white, smooth in outline, and somewhat raised above the surface of the medium. Under the low power they were seen to be darker in the centre than at the margin, and finely granular. The type of growth in various media, using a number of cultures from different animals for comparison, was carefully studied, and proved to be similar in all cases.

Glycerine agar.—24 hours at 37° C. Profuse, grayish-white, moist porcelain-like growth, with wavy edges, distinctly raised above the surface. The water of condensation contained a rather heavy, flocculent sediment, and was markedly viscid. Some tubes showed gas-bubbles in the growth itself, and in all there was gas-formation along the lines of the stab, sometimes in sufficient quantity to raise the whole slant half an inch or more from the bottom of the tube. There was no liquefaction or discoloration of the medium.

Glucose agar.—24 hours at 37° C. The growth was almost exactly like that on glycerine agar, though the gas-formation was more marked.

Gelatin.—5 days at 22° C. The surface growth closely resembled that on agar, but was less profuse. The growth along the stab was slight, and at the point of inoculation there was a raised, nail-head growth, not extending far from the centre. There was no liquefaction nor gas formation.

Litmus milk.—24 hours at 37° C. There was complete coagulation in every case, with acid reaction. The beginning of the acid reaction was noted in about 15 hours from the time of inoculation. During 20 days there was no peptonization.

Bouillon.—24 hours at 37° C. There was diffuse cloudiness, with a heavy grayish-white, flocculent sediment. Later, the bouillon became very viscid, hanging in strings from the loop.

Glucose bouillon.—24 hours at 37° C. Fermentation tubes, containing 1% glucose bouillon, invariably showed gas-formation. Unfortunately the reaction to lactose and saccharose was not tested, as the cultures were inadvertently destroyed during my vacation.

Potato.—24 hours at 37° C. There was a profuse, moist, viscid growth, grayish in color, containing numerous gas-bubbles.

Indol reaction.—Peptone bouillon kept for ten days at 37° C. and tested with 10% sulphuric acid and 1% sodium nitrite for the formation of indol gave a slight positive reaction in all cases.

Morphology and staining reactions in coverslips from artificial media.—Stained specimens showed a pleomorphic organism, most frequently in the form of a short bacillus, with rounded ends. Special stains for capsules gave negative results, except sometimes with coverslips from litmus milk. The organisms stained readily with the aniline dyes, and decolorized rapidly with Gram's stain. Examination by the hanging-drop method failed to show motility.

Exclusion of other organisms.—In the cases which showed marked gaseous emphysema of the organs, control experiments were made by cultures and by animal inoculations, to ascertain the presence or absence of B. aërogenes capsulatus.

Anaërobic cultures in glucose agar were made from the liver and spleen, and kept in an atmosphere of hydrogen in Novy's jars for 48 hours. Examination of the tubes showed growths similar in all respects to those noted above, except that they were somewhat less luxuriant. No organisms answering in any way to the morphology or staining reactions of B. aërogenes capsulatus were seen.

Bouillon suspensions from the emphysematous livers were made and inoculated into the ear veins of rabbits. The animals were killed by a blow on the back of the neck after a short interval, and kept at body temperature for 48 hours. No emphysema or swelling was noticed, and no gas bacilli were found in coverslips. Cultures from the blood and organs of the animals showed the organism described in detail above.

Animal experiments.—Inoculations of 1 cc. of 24-hour cultures in bouillon into the peritoneal cavities of guinea-pigs which had never been exposed to the infection caused death in from 12 to 186 hours, with symptoms similar to those described in animals dying from the original epizootic. At autopsy there was in every case a sero-purulent

peritonitis, and the organs showed marked congestion and cloudy swelling, but none of the experimental cases showed any gaseous emphysema in either subcutaneous tissue or organs.

Further experiments were made on the two animals which had survived the original infection, to find out if any degree of immunity had been conferred by the previous attack. When they had completely recovered from the first infection, 1 cc. of a 24-hour culture of the organism described was injected into the peritoneal cavity of each. The animals showed no signs of illness on the succeeding day, and the inoculation was repeated, in the same manner and quantity. This was done on the third day also, but the animals showed no sign of any discomfort. In order to make sure that the organism had not diminished in virulence, control inoculations were made at the same time, from the same tubes, into guinea-pigs not previously exposed to the infection. These animals died within 24 hours with the same clinical symptoms, post-mortem findings and cultural results as described.

This observation agrees with that of Howard,[1] who found that by starting with small, non-fatal doses both of living and of sterilized cultures and filtrates of bouillon cultures of a bacillus of the B. mucosus capsulatus group, obtained from a case of hæmorrhagic septicæmia in man, both guinea-pigs and rabbits could be accustomed to withstand doses fatal to untreated animals.

Microscopic examination of hardened sections.—Portions of the heart, lungs, liver, spleen and kidneys were removed at each autopsy, and hardened in Zenker's fluid and in alcohol. Sections were stained by the following methods: (1) Hæmatoxylin and eosin; (2) methylene blue and eosin; (3) Van Gieson's picric acid and fuchsin; (4) Weigert's fibrin stain; (5) Lugol's solution.

Sections of the heart showed marked congestion in all, and cloudy swelling in most cases. The lungs showed marked congestion.

The liver showed extensive changes. There was enormous congestion, both of the hepatic and portal systems. The intralobular capillaries were distended so that the liver cells were often separated into bands and small clumps. In a large number of the vessels no abnormal elements were present, but there was much granular detritus, which stained deeply with eosin. Some of the capillaries were irregularly dilated, and their lumina were empty, or at most contained a small amount of granular material. Many of the central veins of the lobules as well as many of the hepatic veins were much wider than normal. Some of them

[1] *Journal of Experimental Medicine*, 1899, iv, p. 164.

contained granular detritus, but most were empty. The liver cells about these distended veins and capillaries were elongated and flattened. This change often affected several rows of liver cells about these spaces. Some of these cavities were very large, and occupied nearly the whole field of a Zeiss DD lens. In some of these abnormally distended vessels, fibrinous thrombi were found, the thrombus often occupying a portion of the lumen, so as to leave an empty space, round or oval in outline. The vascular changes above described were clearly due to the gaseous emphysema found in the gross sections of the organ. Scattered through the sections capillaries were found, containing fibrinous thrombi. The liver cells were cloudy, and often contained large fat drops; the protoplasm was granular, but the nuclei as a rule stained normally.

Scattered through the sections from two cases were areas of varying size, in which there was more or less complete necrosis of the liver cells, which in many places was typical coagulative necrosis. In some places the cell-protoplasm was hyaline in appearance, while the nuclei stained well and showed but little change. In other places both cells and nuclei took a deep eosin stain, though the outlines of the nuclei could be still readily made out. The necrotic portions bore no special relation to the lobules, though perhaps more of them lay at the periphery than at the centres of the lobules. They varied in size from those including only one or two cells to those as large as or larger than a single lobule; these larger ones were often markedly irregular in shape. In some of these areas both veins and capillaries were filled completely with either hyaline or fibrinous thrombi, in some of the latter of which leucocytes were seen. The liver cells in the immediate vicinity of these degenerated areas, especially of the smaller ones, frequently showed dropsical degeneration. In and about these areas there were numerous spaces similar to those noted in the blood-vessels. Sections treated with Lugol's solution failed to show any amyloid reaction.

Sections of the spleen showed marked congestion. There was a diffuse emphysema, and the vessels were distended as in the liver. The cells of the splenic pulp showed no special changes, and no areas of necrosis were seen.

Sections of the kidney showed marked congestion, extensive cloudy swelling and granular degeneration of the epithelial cells of the convoluted tubules. In many places the cell-outlines could not be made out, but the nuclei throughout the sections stained uniformly sharply. In some sections there was diffuse emphysema, and the vessels were dis-

tended as in the liver and spleen. No areas of necrosis or of cell-infiltration could be seen in any section.

Sections from the liver, spleen and kidney, stained by Weigert's method for fibrin, showed small amounts of fibrin in some of the vessels, but nowhere else, and no organisms of any kind.

Sections were stained with eosin and methylene blue to determine the presence of organisms decolorizing with Gram. In the blood-vessels, especially the veins, and in and about the emphysematous areas, numerous bacilli of from 1 μ to 3 μ in length, and about 0.5 μ in width, were seen. They frequently occurred in pairs, and capsules could often be seen.

The fact that they stained with methylene blue but not by Gram's method, as well as their morphology, confirm the results in the cultures, excluding the possibility of the presence of B. aërogenes capsulatus or other bacteria in the emphysematous areas.

The observations recorded in this paper are of especial interest in view of Howard's[2] report in a recent number of this journal of an instance in a human being of general gaseous emphysema with gas-cysts in the brain, formed after death, and due to a member of the B. mucosus capsulatus group (variety "aërogenes" of Strong). He further found that these two bacilli and three other bacilli of the same group and variety as the latter, obtained at autopsy from various lesions, caused general gaseous emphysema in the cadavers of rabbits most abundantly with, but also without, intravenous injections of lactose or glucose solutions before killing the animals.

There are three facts of special importance connected with the observations herein reported:

1. The spontaneous occurrence of a very fatal epizootic among laboratory guinea-pigs due to B. mucosus capsulatus.

2. The development of immunity of a high grade from otherwise fatal doses of the organism causing the epizootic.

3. The development of gaseous emphysema of various organs in 56 per cent of the animals either just after or 24 hours after death, due to the same bacillus, the usual cause of such emphysema, namely B. aërogenes capsulatus, being carefully excluded.

[2] *Journal of Experimental Medicine*, 1900, v, p. 139.

It is to be regretted that by an accident during my absence the cultures of the bacillus were destroyed before I had an opportunity of determining its exact position in the "mucosus capsulatus" group, according to the amount of gas formed in glucose, lactose and saccharose bouillon. There seems to be no doubt, however, that it belongs to Strong's[3] "aërogenes" variety of the B. mucosus capsulatus group, on account of its great production of gas in the animal body, and the rapid coagulation of milk. This view is further supported by Howard's already cited observation in this laboratory of gas-formation in both human and rabbit cadavers, caused by bacilli of this variety.

[3] *Journal of the Boston Society of the Medical Sciences*, 1889, iii, p. 185.

ON THE RELATION OF CHRONIC INTERSTITIAL PANCREATITIS TO THE ISLANDS OF LANGERHANS AND TO DIABETES MELLITUS.

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Plates XXVII and XXVIII.

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INTRODUCTION.

In the pancreas are found certain structures whose nature is still obscure, the architecture of the organ being much more complex than that of the salivary glands which it closely resembles. Within the acini, and apparently in their lumina, are the so-called centro-acinar cells whose origin and function are as yet unexplained. P. Langerhans,[1] in the first important contribution to our knowledge of the histology of the pancreas, described groups of cells situated between the acini and differing markedly from those of the ordinary glandular type. These groups, usually round, are composed of small, irregularly polygonal cells with a round nucleus and homogeneous refractive cell body. Numerous observers have since described these structures, which are usually designated "islands of Langerhans."

[1] Beiträge zur mikroskopischen Anatomie der Bauchspeicheldrüse. Inaug.-Diss. Berlin, 1869.

In injected specimens Kühne and Lea [2] found glomeruli composed of wide tortuous anastomosing capillaries between which lie the cells which Langerhans described. The ducts of the gland are not continued into these bodies.

Various opinions are held concerning the nature of these cell groups. Some observers have thought that they are follicles of lymphatic tissue scattered through the organ. Many believe that they have the same origin as the secreting elements of the gland and, formed during embryological life, persist thereafter and probably subserve some special function. Lewaschew [3] subjected the pancreas to prolonged stimulation by overfeeding or by the repeated administration of pilocarpin, and thought that he was able to transform small groups of acini into typical interacinar islets, thus increasing their number at the expense of the secreting tissue. His experiments have not been confirmed.

It is not surprising that little is known concerning the function of structures whose nature is so little understood. Several writers (Laguesse,[4] Schäfer,[5] Diamare [6]) have suggested that they furnish an internal secretion which influences carbohydrate metabolism. The only evidence in support of this suggestion is contained in the short preliminary notice of Ssobolew,[7] which has appeared since the completion of the present study. He states that after feeding animals on carbohydrates the cells of the islands become more granular. After ligating the duct of Wirsung in dogs the islands of Langerhans, he finds, are not implicated in the sclerotic process which ensues. He thinks that this fact explains the absence of glycosuria after ligation of the duct. In human cases I had observed similar resistance of the islands to the inflammation consequent upon obstruction of the duct. In the pancreas of two diabetics, Ssobolew was unable to discover islands of Langerhans.

[2] Untersuchungen a. d. physiol. Institute d. Univ. Heidelberg, 1882, ii, p. 488.
[3] *Arch. f. mikros. Anat.*, 1886, xxvi, p. 452.
[4] *Compt. rend. Soc. de Biol.*, 1893, 9. s., v, p. 819.
[5] *Lancet*, 1895, ii, p. 321.
[6] *Internat. Monatschr. f. Anat. u. Phys.*, 1899, pp. 155, 177.
[7] *Centralbl. f. allgem. Path. u. path. Anat.*, 1900, xi, p. 202.

From a histological study of these structures in man and in lower animals, in injected specimens, and in glands stimulated by the administration of pilocarpin, I have reached the following conclusions[7a]: (1) The islands of Langerhans are composed of cells having the same origin as those of the glandular acini but forming structures which are independent of the secreting apparatus and in intimate relation with the vascular system. (2) In the splenic end of the cat's pancreas they have a definite position within the lobule, each of which contains one of these structures. (3) In the human pancreas they are more numerous in the splenic extremity or tail than elsewhere. Similar variation in their number is observed in cats and dogs. (4) Prolonged stimulation of the gland does not, as claimed by Lewaschew, transform groups of acini into islands of Langerhans.

Embedded as are these bodies in the substance of the organ, they cannot readily be subjected to experimental conditions which do not equally affect the secreting structures. It is particularly desirable, therefore, to observe what changes they undergo under pathological conditions and, if possible, to bring such alterations into correlation with concomitant pathological phenomena. From such a study, heretofore little pursued, we may hope for suggestions of the function or anatomical relationship of these obscure structures.

Acute rapidly destructive lesions of the pancreas, for example hæmorrhagic pancreatitis, affect the various elements of the gland almost simultaneously, and complete disintegration of greater or less extent results. When the organ is attacked by the less active irritants which produce chronic inflammation the different histological constituents are given greater opportunity to exhibit differences in their ability to withstand the destructive process. The islands of Langerhans do not always show alterations corresponding to those which occur in the tissues of the acini about them, often persisting, though the adjacent parenchyma is destroyed. Moreover, while in some varieties of chronic inflammation they are but little implicated in the sclerotic process, in others they may be markedly affected. It becomes of interest, therefore, to study the relation of these bodies to the various forms of chronic pancreatitis that are distinguishable.

[7a] *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 205.

The histological details of chronic pancreatitis have been, however, little studied and slight attention has been given to the classification of the various types. For the purpose of the present study it is desirable to adopt a classification, although it is based upon the somewhat limited material available.

That form of chronic inflammation which occurs during fetal life and is associated with other manifestations of congenital syphilis presents histological features which distinguish it from the chronic pancreatitis of adult life. It is a disease of the developing organ and may appropriately be first considered.

CONGENITAL SYPHILITIC PANCREATITIS.

Birch-Hirschfeld[8] first drew attention to the frequency with which the pancreas is affected by congenital syphilis, and described the lesion so accurately that nothing had been added to our knowledge of it until the appearance of the recent article of Schlesinger,[9] who has made a systematic study of the condition.

Birch-Hirschfeld found the pancreas affected in 13 of 23 cases of congenital lues, but subsequent observers have found the lesion much less frequently and, indeed, Birch-Hirschfeld,[10] studying a second group of cases, found changes in the organ only 29 times in 124 syphilitic new-born. Schlesinger in six instances found the enlarged firm organ the seat of a diffuse interstitial pancreatitis characterized by proliferation of interlobular and interacinar tissue penetrating at times between the cells of the acini. This inflammatory new growth is followed, he thinks, by atrophy of the parenchymatous elements, which, though they do not exhibit appearances of degeneration, atrophy and disappear. The growth of interstitial tissue, he finds, has its origin about the blood-vessels, and the arteries are the seat of a syphilitic periarteritis, the adventitia being infiltrated with lymphoid cells. As the lesion progresses the capillary network about the acini disappears. Schlesinger has observed that the islands of Langerhans are neither invaded by the new growth of interstitial

[8] *Arch. d. Heilkunde*, 1875, xvi, p. 174.

[9] Virchow's *Archiv*, 1898, cliv, p. 501.

[10] Gerhardt's Handbuch d. Kinderkrankheiten, iv, Abth. 2, p. 753. Tübingen, 1880.

tissue nor implicated in the atrophy which affects the cells of the acini.

The histological details which I have observed in two instances of congenital syphilis of the pancreas will be recorded. The relation of the islands of Langerhans to the inflammatory process is of interest.

Case I.—Infant lived three hours. Length of body 40 cm.

Anatomical diagnosis.—Congenital syphilis; interstitial pneumonia; interstitial pancreatitis; splenic tumor; chronic perisplenitis.

Microscopic examination of pancreas.—The interstitial tissue is greatly increased at the expense of the parenchyma. The lobules, composed of a few acini scattered irregularly in dense cellular stroma, form groups separated by looser cellular tissue in which are situated the small veins and arteries. The smallest ducts, beset with acini along their course, terminate in a group of acini which, though much less numerous than those ordinarily forming a lobule, are of normal size and are composed of cells showing no evidence of degeneration. The interstitial tissue, particularly that between the groups of lobules and hence about the smaller vessels, is very rich in cells, which often form foci of dense cellular infiltration. Cells of lymphoid and of epithelioid type are numerous, but in even greater number, particularly about the blood-vessels, are round, oval or polygonal cells with eccentrically situated nucleus. They have the characteristics of the plasma cells of Unna. Cells with eosinophilic granules are also abundant and are of two types: (a) Mononuclear cells whose protoplasm is closely packed with large conspicuous cosinophilic granulations; (b) small cells whose nucleus is usually bilobed or trilobed; eosinophilic granulations of smaller size scattered throughout the cell body are most abundant about the nucleus.

A conspicuous feature of the histological picture is the presence of compact round masses of cells embedded in the interstitial tissue, which is usually concentrically arranged immediately about them. By the character of the cells which, polygonal in shape, are stained bright pink with eosin and by their arrangement in columns between which are capillary vessels, these structures are identified as the islands of Langerhans. Though they are embedded in the stroma, which separates widely the neighboring acini, they are not invaded by the inflammatory change. At times it is demonstrable, most conveniently in serial sections, that these islands are in continuity with the ducts and acini of the gland (Plate XXVII, Fig. 1). At the periphery of the island one of the columns projects beyond the general circular outline and is continuous with

epithelial cells which, staining less brightly with eosin, are arranged about a lumen and are in turn continuous with adjacent acini. In many instances, however, an island traced through a series of sections is found completely isolated in the fibrous tissue.

CASE II.—Infant lived four hours. Length of body 50 cm.

Anatomical diagnosis.—Congenital syphilis; pemphigus neonatorum; interstitial pneumonia; interstitial hepatitis and pancreatitis; splenic tumor.

Microscopic examination of the pancreas.—The interstitial tissue is greatly increased and the parenchyma is in very great part replaced by it, acini and groups of acini being widely separated. The new tissue is very cellular, but the cells are for the most part of the epithelial type and accumulations of round cells are not found. Plasma cells and cells with eosinophilic granulations are but rarely seen. The acini form small groups which may be regarded as primary lobules, though the acini composing them are much less numerous than those of a normal lobule. Islands of Langerhans are conspicuous as compact round masses of epithelial cells and are scattered abundantly throughout the organ. The fibrous tissue is often concentrically arranged about them and at times they lie completely isolated. Not infrequently, however, as in Case I, they are in continuity with the neighboring acinar tissue; a double row of cells is found to be continuous on the one hand with a cell column of the island, on the other with a small duct.

The preceding cases apparently represent different stages of the syphilitic lesion. In Case I proliferating fixed tissue cells are very abundant, while cells, in part at least of vascular origin, namely, plasma cells and eosinophiles, are numerous and the condition may be interpreted as the active stage of a chronic inflammatory process. In Case II, though interstitial tissue is more abundant and the persistent parenchymatous elements are more scattered, cells of the lymphoid type are few in number, while plasma cells and eosinophiles are almost absent. The process here is more advanced and is no longer active.

A conspicuous feature in both cases is the presence of numerous islands of Langerhans surrounded by newly formed stroma, but uninvaded by it. In many instances the islands are found to be in continuity with the secreting structures of the gland (Plate XXVII, Fig. 1.) A cell column of the island is continuous with a small

duct-like structure, which is in turn continuous with glandular acini. The lumen of the duct does not penetrate into the island.

Birch-Hirschfeld, finding the pancreas of syphilitic fetuses rarely affected unless they had survived the full period of uterine development, came to the conclusion that the condition has its onset during the last months of fetal life. Schlesinger, however, cites the cases of Müller and Mraczek, in which, at the 5th month of development, advanced lesion of the organ occurred, and from his own experience concludes that the pancreas may be affected as early or as late as other organs.

The pancreas arises as an outgrowth from the intestinal canal, and the development of its parenchyma takes place in a mass of mesoblastic stroma which is replaced as the growth of the gland proceeds. At an early period of development, for example at the 5th month of fetal life, the acini form small groups widely separated by embryonic connective tissue. In my two cases of syphilitic pancreatitis the parenchyma presents the appearance observed about the 5th month of development, with the exception that the islands of Langerhans, which are inconspicuous in the undeveloped organ, are marked features in the syphilitic pancreas. In neither of the syphilitic cases was it possible to observe degenerative changes in the cells. The acini form irregular groups containing much fewer members than ordinarily compose a fetal lobule, or, as in the developing organ, form dilatations upon the sides of the small ducts. It is conceivable, therefore, that the disease, like many syphilitic lesions, is one of the interstitial tissue and the changes in the parenchyma result not so much from a destruction of the parenchyma as from an interference with its growth. The similarity between the syphilitic and the undeveloped organ may be thus explained. The development of the individual cell, however, is not retarded and the islands of Langerhans are the result of an early cell-differentiation. In many instances the islands remain in continuity with the tubular structures from which they had their origin. Often, however, the connecting strand of cells is no longer discoverable, and the condition resembles that ordinarily observed in the organ at the end of fetal development.

CHRONIC PANCREATITIS OF THE DEVELOPED ORGAN.

Several types of chronic pancreatitis affecting the fully developed organ have been described, and with the experimental demonstration of a relation between the pancreas and carbohydrate metabolism numerous attempts have been made to distinguish a variety of the lesion constantly associated with diabetes mellitus. A classification of these various forms of chronic inflammation based upon etiological data, though desirable, would be, with our present knowledge, as unsatisfactory as a similar classification of the varieties of hepatic cirrhosis. From an experimental study Carnot[11] reaches the conclusion that pancreatitis may result (*a*) from mechanical cause, for example, obstruction of the pancreatic ducts, from the action (*b*) of toxic material or (*c*) of microorganisms carried to the gland by the blood, or by the lymph or by way of the duct. Such a classification does not aid in the interpretation of lesions observed at autopsy, the etiological factors concerned being in the majority of instances obscure.

In many examples of chronic pancreatitis fibrous tissue between the lobules is increased; in others the interacinar tissue shows marked proliferation; occasionally individual cells are apparently separated by strands of fibrous tissue. Corresponding types of inflammation have been described as interlobular, periacinous, and monocellular.

It has been thought that the increase of interstitial tissue may have at times a constant relation to the blood-vessels or to the ducts, being in part at least a proliferation of the connective tissue about these structures. Lemoine and Lannois[12] have described perivascular interstitial pancreatitis. From a study of four cases of chronic inflammation associated with diabetes they have thought that the new growth of fibrous tissue has its origin in the walls of the blood-vessels. They find about the vessels masses of sclerotic tissue sending processes between the acini and even separating the individual cells (sclérose unicellulaire). G. Hoppe-Seyler[13] has described chronic

[11] *Recherches expérimentales et cliniques sur les pancréatites.* Thèse, Paris, 1898.
[12] *Arch. de méd. expér.*, 1891, iii, p. 33.
[13] *Deutsch. Arch. f. klin. Med.*, 1893, lii, p. 171.

interstitial changes which he thinks are the result of arterial sclerosis. The parenchyma, he believes, undergoes degeneration as a consequence of disturbed nutrition, but no anatomical relation exists between the vessels and the new-formed tissue. Chronic pancreatitis in a case described by Rosenthal[14] was accompanied by what he regards as alterations of the lymph-vessels, "lymphangitis proliferans," indicative, he thinks, of a probable syphilitic origin.

In the instances of chronic inflammation of the pancreas which have been available for my study, no constant relation has been discoverable between the new-formed tissue and the veins, arteries, lymph-vessels, or ducts, and there is no evidence that the process had its origin about these structures. Even where chronic pancreatitis follows obstruction of the ducts, sclerotic tissue is not more abundant about the ducts than elsewhere.

Two types of interstitial inflammation are, however, distinguishable. On the one hand, though the sclerosis is never accurately confined to one locality, it may be conspicuous between the lobules, the intralobular or interacinar tissue being little, if at all, increased. On the other hand the interlobular tissue may be only slightly altered, while fibrous tissue which replaces the parenchyma separates individual acini. In the first case the lobulation of the gland, which is normally obscure, becomes more conspicuous and wide bands of sclerotic tissue separate groups of lobules. The lobules are invaded in greater or less degree by the newly formed stroma and often entire lobules are in process of disintegration and replacement, but the progress of the lesion has been apparently inward from the periphery of the lobule. With the second type of chronic inflammation the lobulation of the gland is not accentuated, and the new fibrous tissue, primarily within the lobule, has a diffuse character, a network of irregular fibrous strands which vary much in thickness containing the glandular acini in its meshes.

The two types of chronic interstitial inflammation—(*a*) interlobular and (*b*) interacinar—characterized by the primary localization of the lesion, present other histological peculiarities. Of present interest is

[14] *Zeitsch. f. klin. Med.*, 1892, xxi, p. 401.

the different relation which they bear to the islands of Langerhans, and it is desirable to study separately the changes affecting these bodies in the two conditions. The cases which have been studied exhibit individual differences and in a few instances the histological details will be briefly described.

CHRONIC INTERLOBULAR PANCREATITIS.

The sclerosis of the gland which follows obstruction of the ducts belongs to the interlobular type. Its definite etiology, as well as certain histological features, serve to distinguish it from other varieties of chronic pancreatitis whose etiology is more obscure. I shall first consider chronic inflammation of the interlobular type not caused by duct-obstruction. Six examples of this form of inflammation have been studied. Chronic inflammation, the result of duct-obstruction, will be subsequently considered.

In two cases (III and IV) the increase of interstitial tissue is moderate in amount and is most marked between the lobules, defining them more clearly than usual. Accumulations of lymphoid cells, among which are plasma cells and eosinophiles, indicate that the inflammatory process is still active. The lumina of the acini are widely distended, the secreting cells are much flattened and often show marked alterations; the scant protoplasm may no longer present a basal zone which stains with nuclear dyes, *e. g.*, methylene blue or hæmatoxylin, and the nucleus is often much swollen and irregular in shape and stains faintly. The islands of Langerhans are not the seat of similar changes; the cells composing them are normal in appearance, and though the acini are often separated by strands of sclerotic tissue, the islands are rarely, if ever, penetrated by this tissue.

In a third case (V) acute inflammation is associated with beginning proliferation of the interstitial tissue. The gland acini are distended and contain bacilli, morphologically of the colon type, and inflammatory products. The islands of Langerhans are apparently normal.

These three cases serve to direct attention to the fact that though the secreting acini have undergone marked degenerative changes, the islands of Langerhans may be unaltered. Anatomical peculiarities may explain the greater resistance of the interacinar structures to the

inflammatory process: (1) The vascular supply of the islands is richer than that of the adjacent acini. (2) Since the ducts do not penetrate them they are less exposed to the action of irritants which reach the gland by way of the duct.

In a fourth case of interlobular pancreatitis (Case VI) new fibrous tissue poor in cells outlines more conspicuously than is usual the gland lobules. The new-formed tissue penetrates the lobules and forms an intralobular network whose meshes are narrowest next the interlobular bands. Here the acini are atrophic in appearance. The islands of Langerhans surrounded by acini are, when the lobules are well defined, usually situated near their centre. The islands are therefore surrounded by the least changed acini and are themselves unaltered. Their very unusual abundance may be explained by the change in the parenchyma between them. The tissue being diminished in volume as a result of partial replacement by fibrous tissue, they are brought closer together and are more numerous in a given area.

Two cases (VII and VIII) represent advanced perilobular sclerosis. Wide bands of fibrous tissue, often containing lymphoid cells in great number, separate groups of lobules and send processes of new tissue into them. In places the glandular tissue is fairly well preserved, while elsewhere entire lobules are found in process of disintegration, the dilated and atrophied acini being separated by strands of fibrous tissue. The islands of Langerhans, scattered more abundantly than usual in the parenchyma and unaffected by the process, are surrounded by a delicate connective-tissue outline and are penetrated by delicate capillaries. Occasionally one finds an island surrounded by a few scattered acini, and though the neighboring secreting tissue has been almost entirely replaced by interstitial tissue, the island remains intact.

Chronic Pancreatitis Following Obstruction of the Ducts.

It has long been known that occlusion of the pancreatic ducts causes chronic interstitial inflammation of the gland. In human cases the usual causes of such obstruction are calculi or new growths, especially carcinoma, and numerous instances of pancreatitis follow-

ing such lesions are recorded. A large number of experimenters have produced sclerosis in animals by ligating the ducts.

Arnozan and Vaillard[15] have studied the progress of the lesion in rabbits. The ducts soon become dilated, their epithelium proliferates, and cells are desquamated into the lumen. At the end of twenty-four hours the protoplasm of the secreting cells becomes clearer and the nucleus stains more deeply with carmine. At the end of about four days the swollen nuclei may almost completely fill the cell. At the end of seven to nine days round cells are numerous and before the fourteenth day connective tissue in large part replaces the parenchyma. The authors think that sclerosis is caused by the ferment present in the retained secretion.

Carnot[16] produced interstitial inflammation by injecting the ferment papain into the duct. He thinks that several factors are active in the production of the sclerosis which follows obstruction of the ducts. The retained secretion, he believes, has a toxic action upon the parenchymatous cells. Obstruction to the outflow of fluid favors extension of infection along the duct from the duodenum. The action of infection plays an important part when occlusion is caused by calculus formation, a process which he thinks is of bacterial origin. Carnot suggests, moreover, that alterations of the reflex nervous stimuli which reach the obstructed gland are etiological factors in producing atrophy of the parenchyma. Such stimuli are no longer capable of exciting normal functional activity and, he thinks, cease to exert their influence on the metabolism of the secreting cells. The atrophy of the parenchyma may be somewhat analagous to the muscular atrophy which follows section of motor nerves.

Since the cells forming the islands of Langerhans have no communication with the lumen of the ducts and, presumably, play no part in producing the pancreatic juice, the possible factors mentioned would, if active, affect primarily the acini and only secondarily, if at all, the interacinar islands.

In the cases which I have studied varying degrees of atrophy and sclerosis have followed partial or complete occlusion of the pancreatic

[15] *Arch. d. phys. norm. et path.*, 1884, 3. s., iii., p. 287.
[16] Op. cit.

ducts. In two cases (IX and X) carcinoma of the gland was associated with chronic pancreatitis due to obstruction of the duct. In the first case (IX) retention cysts are numerous and changes are slight except in the immediate neighborhood of the largest of the cysts where dense fibrous stroma has replaced the glandular tissue. Dilated acini composed of flat atrophied cells are the only remnants of secreting tissue, while here and there are groups of epithelial cells, not differing from the islands of Langerhans present in the neighboring relatively normal parenchyma; they have withstood the sclerotic process. In the second case (X) the new growth has invaded the body of the pancreas and that part of the gland which is distal to the invading growth is alone sclerotic. Here the occurrence of an active chronic inflammatory process is shown by the presence of numerous lymphoid cells, plasma cells and eosinophiles in the interstitial tissue. Cell changes similar to those described by Arnozan and Vaillard are demonstrable. The most marked increase of fibrous tissue is between the lobules, but acini showing marked atrophic changes are often widely separated by new tissue. The structure of the islands of Langerhans is, however, unaltered.

In the following cases advanced chronic interstitial pancreatitis has followed obstruction of the pancreatic ducts:

Case XI.—*Summary of clinical history.*—Female; aged 60 years.

Illness began about one year before death with symptoms of obstruction of the common bile-duct. At operation performed by Dr. Halsted [17] a carcinoma of the bile-papilla and diverticulum of Vater was found and removed. The biliary and pancreatic ducts were transplanted into the duodenum. Subsequently an anastomosis was made between the gall-bladder and duodenum.

Anatomical diagnosis.—Recurrent carcinoma of the duodenum; metastasis in liver; occlusion of pancreatic duct; chronic interstitial pancreatitis; biliary fistula.

Pancreas.—On the left lateral wall of the duodenum is a crater-like ulcer with raised edges abutting upon the head of the pancreas. The pancreatic duct is included in the carcinomatous tissue at the base of the ulcer. The duct is greatly dilated and the pancreas is small and sclerotic.

[17] This case is described by Dr. Halsted in the *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 4.

Microscopic examination of pancreas.—The parenchyma of the head and body has been almost completely replaced by dense fibrous tissue which contains fat in considerable quantity. Small isolated masses of glandular tissue still persist and are subdivided by penetrating strands of fibrous tissue. The stroma is in great part very dense and poor in cells. Here and there, however, round cells of the lymphoid type form large accumulations and mingled with them plasma cells are very numerous. Cells with eosinophilic granulations are present in the periphery of such foci and in the dense fibrous bands as well. The small ducts are dilated. The glandular tissue is in part normal in appearance, the gland-cells being little affected by the sclerotic process. In other situations secreting tissue is undergoing disintegration and the connective tissue not infrequently marks out areas which correspond apparently to lobules, but contain only a few atrophied acini composed of flattened cells about a dilated lumen. Here the inflammatory process is active; the connective tissue separating the atrophic acini is very cellular and contains many lymphoid cells, plasma cells and eosinophiles.

Islands of Langerhans, more abundant in the tail and body than in the head, are present in the relatively normal glandular tissue and are unaltered (Plate XXVII, Fig. 2). One not infrequently sees an island situated in the centre of a lobule which is undergoing disintegration. Scattered atrophic acini are separated by interstitial tissue containing large numbers of proliferating or exuded cells, but the island is normal in appearance and is not invaded by the newly formed fibrous tissue which surrounds it and isolates the much changed acini.

About an unaltered island may be found only a few acini to indicate that it was formerly embedded in the parenchyma, while elsewhere in the dense fibrous bands are seen isolated structures whose cells do not differ in character or arrangement from those of the interacinar islets. Such islands though surrounded by sclerotic tissue are not invaded by it and their cells which are normal in appearance form columns separated by delicate capillary vessels.

These isolated islands, however, finally undergo degenerative changes. They are diminished in size and often distorted. The cells, particularly at the periphery, crowded together, become smaller, and their nucleus also smaller than usual is often irregular in shape and is stained very deeply. Further changes are followed with difficulty since the much altered groups of cells are hardly recognizable as islands. Small groups of epithelial cells separated by strands of connective tissue probably represent a late stage of atrophy and precede final disappearance and replacement by fibrous tissue.

Case XII.—*Summary of clinical history.*—Male; aged 43 years. Diagnosis: Pulmonary tuberculosis. No symptoms of diabetes were noted.

Anatomical diagnosis.—Chronic pulmonary tuberculosis; gelatinous and caseous pneumonia; miliary tubercles; tuberculous pleurisy. Cirrhosis of the liver with fatty degeneration. Parenchymatous and fatty degeneration of the kidneys. Splenic tumor. Pancreatic calculi; interstitial pancreatitis; parapancreatic fat necrosis.

Pancreas.—The duct of Wirsung is much distended by numerous calculi. The small ducts are also dilated and filled with fine gritty material. The gland-tissue has in large part disappeared and is replaced by interstitial tissue containing much fat in which are small opaque white areas of necrosis. The concretions give the reactions of calcium carbonate.

Microscopic examination of pancreas.—The parenchyma has been in very great part replaced by dense sclerotic tissue in which are scattered foci of round cells. The ducts are widely dilated and contain clumps of calcareous material. In the head of the organ are small areas of glandular tissue subdivided by interlobular strands of fibrous tissue which occasionally send projections between the acini. Occasionally gland-lobules are found in process of disintegration, tubular atrophied acini with dilated lumen being separated by new-formed interstitial tissue. Scattered in the sclerotic tissue, most abundant in a section from the splenic end of the organ, are round and oval clumps of cells arranged in columns between which, though the tissue about is densely fibrous, one sees delicate capillaries, often distended with red blood-corpuscles. These bodies, which have the characteristic structure of islands of Langerhans, present no similarity to secreting tissue in process of destruction. Occasionally an island has the appearance of being compressed and distorted.

Case XIII.—*Summary of clinical history.*—Male; aged 50 years. The patient gives a history of alcoholic excess. His illness began five months before its fatal termination with symptoms of pulmonary tuberculosis which gradually increased in intensity. On admission to the hospital two months after the onset of his illness the urine contained 5.2% of sugar. When given a diet very poor in carbohydrates (v. Noorden's standard diabetic diet) sugar disappeared from the urine and reappeared only when carbohydrates were added—90 grms. of white bread to the daily diet.

Anatomical diagnosis.—Pancreatic calculi; chronic interstitial pancreatitis. Chronic pulmonary tuberculosis with cavities. Chronic dif-

fuse nephritis; large white kidneys. Anthracosis of lungs, spleen, and kidneys.

Pancreas.—The organ, which is intimately united to the adjacent structures, is much diminished in size and is tough and fibrous in consistence. The atrophy is most marked in its central part, which forms a narrow isthmus connecting the head and tail and consists of the duct and a small amount of fibrous tissue about it. The duct, slightly enlarged, contains viscid white fluid and a number of small, granular, gritty, yellow calculi, the largest being the size of a split pea. On section the tissue has a grayish-yellow color and small masses of parenchyma project prominently between thick bands of connective tissue. Minute opaque points suggest fat necroses.

Microscopic examination of the pancreas.—Sclerosis is far advanced and is most marked in the tail of the organ where glandular acini are almost entirely absent. The glandular tissue which persists occurs as compact masses rarely more than 2 or 3 mm. in diameter embedded in dense stroma. At the periphery of this relatively normal parenchyma are found lobules or portions of lobules in process of disintegration and replacement by the interstitial tissue. Here acini composed of low cells about a dilated lumen are scattered in the fibrous stroma. The fibrous tissue is in general poor in cells, but in many places, particularly about the large ducts, lymphoid and plasma cells are abundant.

In the masses of glandular tissue islands of Langerhans are present, and though of small size, are normal in appearance. Very numerous, particularly in the dense stroma of the body and tail, are masses of polygonal cells occupying conspicuous, sharply outlined, round or oval spaces in the sclerotic tissue. They do not differ from the islands of Langerhans found elsewhere. They are often situated almost side by side, separated by only a small amount of stroma, so that at times ten or twelve are seen in the field of the low power. Examination readily shows that they do not represent groups of acini in process of destruction. Very frequently in the fibrous tissue about these islands lymphoid cells and plasma cells are very numerous and it may be assumed that the inflammatory process is still active.

The persistent islands are finally involved in the general sclerosis. An increase of fibrous tissue occurs along their capillaries which become coarse strands subdividing the body into small masses of atrophied cells. One finds broad bands of dense fibrous tissue containing no epithelial elements or only an occasional compressed group of cells similar to those forming the islands.

In the preceding cases (XI, XII and XIII) advanced chronic pancreatitis has followed obstruction of the ducts. The organ is densely sclerotic, glandular tissue having been replaced in very large part by fibrous stroma. Small masses of relatively well-preserved parenchyma, little if at all invaded, are embedded in fibrous tissue which contains almost no epithelial elements. Areas are seen where disintegration of the glandular substance is in active progress and here lymphoid cells are present in large numbers. A striking feature of the process is the abundance of the plasma cells of Unna, among which are cells with eosinophilic granulations.

The scattered acini show atrophic changes similar to those previously described. The islands of Langerhans which occur in this altered glandular tissue are unchanged and, even though the neighboring acini are widely separated by inflammatory new growth, are uninvaded (Plate XXVII, Fig. 2). The secreting tissue about them finally disappears and they remain completely isolated in the stroma, not infrequently the only vestiges of parenchymatous tissue in wide sclerotic bands. In a section from such an area, isolated islands may be very numerous, and since the sclerotic tissue occupies less space than the acini which it has replaced, they appear to be much more abundant than in the normal glandular parenchyma.

As it is improbable that the vessels supplying the islands with blood remain unchanged in the indurated stroma, it can hardly be doubted that the nutrition of the cells suffers. The tissue growing older apparently contracts and compresses them; their cells become smaller, the nuclei are small, irregular, and stain deeply. They finally disappear, being replaced by fibrous tissue, which may contain an occasional isolated group of much atrophied cells or may be completely devoid of such structures.

The islands of Langerhans resist the sclerotic process which follows the damming back of secretion upon the gland, and finally suffer only when the acini are almost entirely destroyed and replaced by dense scar-like tissue. This is what we might expect when we consider that since the lumen of the duct is not continued into the islands it is hardly conceivable that they are concerned in the production of the

pancreatic juice, so that they are not exposed to its injurious action when the outflow is obstructed. The changes which the isolated islands undergo are, it appears, due to compression by the contracting scar-like tissue in which they are embedded and to alterations of their blood-vessels. Doubtless the rich vascular system of the parenchyma is in large part obliterated when the acini are replaced by interstitial tissue and, consequently, the network of vessels within the island, which freely anastomose with the adjacent capillaries, is, as the process advances, less freely supplied with blood.

CHRONIC INTERACINAR PANCREATITIS.

The type of pancreatitis, which may be conveniently designated "interacinar," is characterized by the presence of new-formed tissue within the lobules. The lesion is diffuse but somewhat irregular in distribution; at one point there may be a general thickening of the connective-tissue network supporting the acini, while elsewhere occur compact bands or small masses of stroma. Though the interlobular tissue is not unaffected by the inflammatory change, its proliferation is not a constant feature of the histological picture. The lobulation of the gland is not accentuated as with the interlobular type, but, on the contrary, is obscured, since masses and strands of new tissue within the lobules make inconspicuous the interlobular boundaries. This type is much less common than the perilobular form, and I have been able to study it in only three cases. One of these was associated with the condition of general pigmentation to which von Recklinghausen[18] gave the name hæmochromatosis, and, differing slightly from the other two, it will be considered separately.

Case XIV.—*Summary of clinical history.*—The patient gives no history of alcoholic excess. The present illness began twenty months before death with polyuria. Much body weight has been lost. A year and a half before death the spleen was palpable and hæmatemesis occurred at intervals. At this time the urine contained 3.5 to 3.8% of sugar. The patient was readmitted to the hospital four days before

[18] *Tageblatt der 62 Versammlung deutscher Naturforscher und Aerzte in Heidelberg*, 1889, p. 324.

his death with ascites and dilated superficial abdominal veins. The urine contained 2.5% of sugar.

Anatomical diagnosis.—Chronic interstitial pancreatitis. Cirrhosis of the liver. Thrombosis of the portal, splenic and mesenteric veins; hæmorrhagic infarction of the intestine. Acute serofibrinous and purulent peritonitis. Acute splenic tumor.

Pancreas.—The organ is small and firm in consistence.

Microscopic examination of the pancreas.—The interstitial tissue is greatly increased and is richly infiltrated with fat. Almost every acinus is in greater or less degree surrounded by fibrous tissue, but the lobulation of the parenchyma is not more distinct than usual. Details of structure are somewhat obscured by partial post-mortem change, but the distribution of fibrous tissue is clearly demonstrable particularly in sections stained with phosphomolybdic-acid hæmatoxylin (Ribbert's method). The connective tissue forms a network in whose meshes lie the acini; the coarseness of the fibrous strands and the size of the meshes which they form vary much and correspond to the greater or less alteration of the contained acini. In many places the glandular tissue of a limited area is almost completely replaced, being represented only by widely separated atrophic acini. The new growth of tissue, which is often conspicuous about the ducts and blood-vessels, bears no constant relation to these structures.

Islands of Langerhans are very abundant and are sharply outlined in sections stained with phosphomolybdic-acid hæmatoxylin, since fibrous tissue, concentrically arranged, forms coarse capsules, separating them from adjacent acini. There is, moreover, a proliferation of the connective tissue within them. Along the capillaries somewhat irregular, spindle-shaped, or elongated nuclei are more numerous than usual, and there is an increased amount of fibrillated material which gives the staining reactions of white fibres. The cells of the islands are often very small and their nuclei, diminished in size, stain deeply. They are closely packed together to form wide irregular columns. Not infrequently the interacinar fibrous tissue is much more abundant in the immediate neighborhood of the islands than elsewhere and here forms a close network of coarse strands with small meshes containing atrophied acini.

Case XV.—*Summary of clinical history.*—Male; aged 47 years. The patient has used alcohol in excess. His health has been good until six months before death. On several occasions he has vomited blood. For three months the appetite has been poor, but thirst has been excessive;

polyuria has been present. The body weight has been fairly well retained. The patient was in the hospital five days preceding his death, during which time the urine contained 0.6 to 2.46% of sugar; acetone was present. He was dull, drowsy and at times delirious.

Anatomical diagnosis.—Chronic interstitial pancreatitis. Cirrhosis of the liver. Chronic passive congestion of the spleen. Ascites. Parapancreatic fat necrosis. Arterial sclerosis. Gangrene of the leg.

Pancreas.—Weight, 108 grms. The organ is firm, particularly at its splenic end. Here the lobulation is obscured, the texture of the gland-tissue is compact, and on careful examination of the cut surface minute opaque points are seen. In the fat within and about the organ are small opaque yellowish-white areas.

Microscopic examination of the pancreas.—Throughout the organ there is an abundant diffuse new growth of interstitial tissue which bears no constant relation to the blood-vessels or ducts or to the interlobular tissue but is between the individual acini. This new tissue is poor in cells and those which it contains have elongated spindle-shaped nuclei. It consists in great part of white fibres loosely packed together. In the meshes of the irregular network which it forms lie acini or small groups of acini which are often atrophic in appearance. Acini of large size containing many centro-acinar cells are seen.

In the tail, islands of Langerhans are abundant and often of very large size, corresponding apparently to the opaque points seen macroscopically. They are surrounded by new fibrous tissue which often forms a thick capsule and separates them widely from adjacent acini. They are, moreover, invaded by the new tissue which often forms coarse ingrowths along their capillaries (Plate XXVIII, Fig. 3). Elongated and spindle-shaped nuclei are somewhat increased in number, but the perivascular thickening is in great part produced by fibrillated interstitial material which, like white fibres elsewhere, stains with phosphomolybdic-acid hæmatoxylin and with acid fuchsin. All the islands are surrounded by dense sclerotic tissue, but some are only slightly invaded by the process. Where there is marked thickening about the capillary vessels, the epithelial cells are diminished in size and are closely packed together; the nuclei are small and stain deeply.

While with the interlobular type of chronic interstitial inflammation the islands of Langerhans are unaffected by the sclerosis and show changes only when the lesion has reached a very advanced stage, in the cases just recorded a new growth of tissue within the lobules and

between the acini invades the interacinar cell-islands. They are almost constantly surrounded by fibrous tissue, which forms, as it were, a capsule separating them from adjacent acini, which are themselves abnormally separated from one another. About the capillaries there is a proliferation of interstitial tissue forming coarse strands between the columns of cells.

In certain instances of the interlobular type, proliferation of interstitial tissue occurs between the acini, but is confined to the periphery of the lobule (Case VI). The islands of Langerhans, situated in the midst of the secreting tissue, often near the centre of the more or less clearly defined lobule, are surrounded by the least changed acini. The condition present in the interacinar type of sclerosis is of different character. In the immediate neighborhood of the island may be found the greatest proliferation of fibrous stroma and the acini, separated from it and from one another by coarse strands of white fibrous tissue, are more atrophic than those at a greater distance. When the inflammatory process affects primarily the periphery of the lobule and progresses toward the centre, the islands are affected only when the lesion is very advanced. When the change occurs diffusely within the lobule all parts are equally affected and the islands suffer in common with the acini. Indeed, it often appears that the favorite seat of the lesion is the immediate neighborhood of the bodies.

Chronic Pancreatitis Associated with Hæmochromatosis.

Chronic pancreatitis associated with the condition which von Recklinghausen described as hæmochromatosis was found, in the only case available for examination, to belong to the interacinar type.

In this disease an iron-containing pigment derived from the hæmoglobin of the blood is deposited in various cells of the body. The seat of most abundant pigmentation is the glandular organs, notably the liver, and hepatic cirrhosis is constantly associated with the condition. As the process advances chronic interstitial inflammation of the other organs ensues. The increasing accumulation of pigment acts injuriously upon the cells and finally causes their disintegration. Fibrous tissue replaces the destroyed cells and contains the pigment set free by their death. Pigment accumulation followed

by cell-death and chronic inflammatory reaction is readily observable in the pancreas to be described.

CASE XVI.[19]—Male; aged 55 years.

Clinical diagnosis.—Typhoid fever. The skin showed a deep bronze-like pigmentation. Glycosuria was not present.

Anatomical diagnosis.—Typhoid fever; ulcers in the ileum; bronchopneumonia. Hæmochromatosis; pigmentation of the liver, pancreas, heart, stomach, intestine, peritoneum, lymphatic glands, skin and testicles. Cirrhosis of the liver. Chronic interstitial pancreatitis.

Pancreas.—Weight 170 grms. The organ is firm in consistence. The cut surface has a uniform deep chocolate-brown color. Septa of fibrous and adipose tissue penetrate the gland.

Microscopic examination of the pancreas.—Interstitial tissue is much increased; in many places it defines the lobules, but as a rule it is diffusely distributed, occurring as irregular masses and strands separating small groups of acini or individual acini. A conspicuous feature is the presence of brown-yellow pigment giving the microchemical reactions of iron both in the gland-cells and in the interstitial tissue. The cells of the acini contain the pigment in varying amount; here and there are seen acini whose cells are distended with pigment-granules, their protoplasm being almost entirely replaced. Such cells often show evidence of degeneration; at times the nucleus has an irregular outline and stains very palely, while in many instances no nucleus is demonstrable. The fibrous tissue replacing the disintegrated cells contains free granules of pigment which are larger and more globular than those within the cells.

Islands of Langerhans are fairly abundant throughout the organ, but are most numerous in sections from the tail. They are constantly surrounded by a small area of fibrous tissue containing pigment in considerable quantity. Embedded in stroma they no longer possess a regular round or oval outline, but are irregular in shape and are penetrated by thickened fibrous strands which follow the capillary vessels. The cells, forming compact columns, contain numerous pigment-granules which when least abundant are situated in the portion of the cell most distant from the capillaries and hence tend to occupy the mid-line of the cell columns. The cells of the islands usually contain much more pigment than those of the adjacent acini. In preparations hardened in Flem-

[19] I have reported this case more fully in *The Journal of Experimental Medicine*, 1899, iv, p. 279.

ming's solution fat can be found in many of the secreting cells, but is constantly present in the cells of the interacinar islets.

The new-formed fibrous tissue is diffusely distributed and bears no constant relation to the lobules. The lesion affects primarily the parenchymatous cells and shows no special tendency to involve those in the periphery of the lobule. The stroma, irregularly thickened between the acini, is constantly increased in the immediate vicinity of the interacinar cell-groups.

The alterations of the islands of Langerhans associated with the deposition of an iron-containing pigment, hæmosiderin, in the parenchymatous cells are as follows: (1) Pigment is abundant in the cells and tends to accumulate in that part which is most distant from the capillaries. (2) The cells undergo fatty degeneration. (3) The island is embedded in a capsule-like mass of fibrous tissue containing pigment granules. (4) Strands of similar tissue penetrate the island, following its capillaries.

HYALINE DEGENERATION OF THE PANCREAS.

In the study of lesions of the pancreas the greatest interest centres in their relation to the disease diabetes mellitus. Before discussing the possible relationship of changes affecting the islands of Langerhans to this disease, I shall describe a very remarkable lesion of the organ occurring in a girl, who, for two years before death, had suffered from diabetes. For the tissues from the case I am indebted to Dr. Flexner, who has kindly placed them at my disposal.

The pancreas is the seat of a lesion which obliterates the vascular supply of a considerable proportion of the parenchyma. Of special interest is the fact that the process, though not confined to the islands of Langerhans, has so completely altered them that in no part of the gland are they recognizable. That intact islands are not discoverable is surprising when we find a considerable proportion of the parenchyma very slightly changed.

Case XVII.—*Summary of clinical history.*—Female; aged 17 years. As a child the patient has never been healthy and when 17 months old her parents state that she suffered with an abscess of the abdominal wall

near the liver. The onset of symptoms of the fatal illness occurred two years before death with extreme thirst and polyuria; sugar was found in the urine and has been constantly present in large amount until death. Record of the quantity has not been preserved. Upon diabetic diet the sugar diminished in amount but did not disappear. Marked loss of body-weight was not noted. Death occurred with coma which appeared suddenly and lasted hardly more than twenty-four hours.

Autopsy.—The only lesion noted was that affecting the pancreas. The entire organ was preserved for microscopic study.

Microscopic examination of the pancreas.—The organ is in large part self-digested and stained specimens have a blurred appearance, cell protoplasm and nuclei staining with almost equal intensity. In the tail, however, several areas where the tissue is well preserved give a clear histological picture of the lesions which are present. The interstitial tissue is increased only in localized areas. Throughout the organ, readily distinguishable even in the most digested portions of the gland, are very conspicuous, sharply defined, round, or oval, hyaline areas embedded in the parenchyma. They vary considerably in size. Where the parenchyma stains deeply with hæmatoxylin these bodies stand out conspicuously as almost completely unstained areas formed by a congeries of tortuous hyaline columns between which are compressed lines of cells apparently of parenchymatous origin.

Much clearer pictures are obtained in sections from the tail of the gland where self-digestion is least advanced. Here in preparations stained with hæmatoxylin and eosin these structures form sharply defined areas (Plate XXVIII, Fig. 4), taking a bright eosin stain in marked contrast to the general ground of glandular tissue, which contains many nuclei staining deeply. Their structure is as follows: Coarse, tortuous, hyaline columns separate strands of tissue, containing nuclei and representing in part at least capillary endothelium, from compressed rows of epithelial cells, evidently atrophied parenchymatous cells. The hyaline material lies immediately outside the capillary wall, between capillary and parenchyma. Occasionally the lumen of the capillary is visible and may contain shadows of red corpuscles.

The hyaline material has at times an indistinctly striated appearance, the striation being parallel to the course of the capillaries. A zone near the capillary endothelium, but not in immediate contact with it, often contains a deposit of calcium salts and stains deeply with hæmatoxylin. The epithelial cells between the tortuous hyaline columns form compressed rows varying in width. The cell-bodies are diminished in size and at times are hardly recognizable. The cells are usually arranged in

columns giving no indication of acinar arrangement, but rarely within such an area or more frequently at its periphery is found a double row of cells about a well-marked lumen.

The hyaline material does not stain by Weigert's method for the staining of fibrin. Reactions for amyloid were not obtained with specimens hardened in alcohol. When sections are stained with phosphomolybdic-acid hæmatoxylin, the hyaline takes a peculiar bright-blue stain in marked contrast to the deep blue-black of the fibrous tissue.

In general the parenchyma in which the hyaline masses lie is not markedly changed. The cells are somewhat smaller than usual and in material hardened in Flemming's solution are found to contain numerous fat droplets. The interstitial tissue is not as a rule increased. In the tail the parenchyma, representing several groups of lobules, has been almost completely replaced by the hyaline structures described, between which is fibrous tissue containing only a few atrophied acini composed of low cubical cells about a distinct lumen. Islands of Langerhans of normal structure are not found. The blood-vessels outside the hyaline areas show no change.

Microscopic examination of other organs.—The liver is normal in appearance; there is no increase of interstitial tissue and the blood-vessels are normal. In a section of the kidney a small collection of lymphoid cells is present at one point. Otherwise no change is noted.

The very remarkable lesion just described has apparently obstructed the vascular supply of a very large proportion of the gland-parenchyma. New-formed hyaline material is deposited between the capillaries and the parenchyma-cells (Plate XXVIII, Fig. 4). This material has a homogeneous hyaline appearance and stains deeply with acid dyes. The tissue which was studied was hardened in 95 per cent alcohol and the absence of reactions for amyloid was not conclusive. That the lesion is not this form of degeneration is shown by the absence of similar change in other organs which, much more frequently than the pancreas, are the seat of amyloid degeneration. I have found in the literature no reference to a similar lesion of the gland.

In the tail of the pancreas areas of hyaline transformation are larger and more numerous than elsewhere, involving at least two-thirds of the sectional area. Though the remainder of the parenchyma is in a fair state of preservation, islands of Langerhans are

not found. This fact is especially remarkable when we remember that the interacinar islets are normally most abundant in this part of the organ. It is evident, therefore, that the lesion implicates these structures, but that it is not confined to them is shown by the extent and abundance of the affected areas. Often they correspond in size and shape to the islands, but they may be several times as large. The occurrence of epithelial cells arranged about a lumen, particularly at the periphery of the altered tissue, shows that acini as well as interacinar islets are affected. In the head and body of the gland, areas of hyaline transformation are less abundant and smaller, usually corresponding in size to islands of Langerhans. Unfortunately, self-digestion of these parts of the organ prevents the recognition of very early stages of the lesion and their relation to the various histological elements.

Of present importance is the fact that the islands of Langerhans are destroyed, or at least isolated from their vascular supply, while a considerable part of the secreting parenchyma is not markedly changed. The occurrence of diabetes mellitus under these conditions is of interest and will be now discussed.

DIABETES MELLITUS.

The great amount of experimental and clinical study which has been devoted to glycosuria and diabetes has brought forth few hypotheses which are not still disputed. That the pancreas has an important influence on carbohydrate metabolism is no longer denied, and it is generally accepted that complete destruction of the organ in animals causes diabetes. The following facts concern the present study:—

(1) Extirpation of the pancreas in animals is followed by symptoms which are characteristic of diabetes mellitus in man. Extirpation of a very large proportion of the organ, less than an eighth or a twelfth remaining (Minkowski), is followed by diabetes of greater or less severity. That man is not an exception to this general rule is shown by a limited number of cases where, following operative removal of a portion of the organ, diabetes has ensued.

(2) Diabetes mellitus is in a considerable proportion of cases accom-

panied by diseases of the pancreas, chronic interstitial inflammation or, less frequently, acute inflammation. The frequent association of two relatively uncommon conditions is evidence that they bear some definite relation to one another.

(3) There is no evidence that pancreatitis is caused by the diabetic condition, and when diabetes accompanies changes in the gland which follow obstruction of the duct by calculi or are associated with malignant growth, conditions certainly not consequent upon diabetes, there can be no reasonable doubt, in view of experimental results, that diabetes is secondary and caused by the lesion of the pancreas. The same conclusion may be reached when chronic interstitial inflammation of the pancreas of obscure etiology is associated with diabetes.

It is well known that diabetes is not always associated with a demonstrable lesion of the pancreas. In the production of glycosuria many factors doubtless take part, and in the present immature knowledge of carbohydrate metabolism it is impossible to maintain that diabetes is always caused by disease of the pancreas. Nevertheless, the experimental and clinical evidence is sufficient to justify the assumption that when diabetes is associated with a destructive lesion of the pancreas, the latter is the cause of the first-named condition.

Chronic pancreatitis is not always or, indeed, in the majority of instances, accompanied by diabetes. Since experimental investigations have shown that in order to produce glycosuria it is necessary to remove a large proportion of the pancreas, we need not expect the condition unless a great part of the parenchyma has been destroyed or functionally impaired.

Various observers have attempted to define a type of pancreatitis peculiar to diabetes. G. Hoppe-Seyler[20] and Fleiner[21] have described cases of the disease in which chronic interstitial inflammation of the organ accompanied general arterial sclerosis. Both writers think that changes in the vessels are followed by nutritive disturbances which cause degeneration of the parenchyma and its replacement by fibrous tissue. The condition, Fleiner suggests, is analogous

[20] Op. cit.

[21] *Berl. klin. Wochenschr.*, 1894, xxxi, pp. 5, 38.

to the contracted kidney which is at times associated with general arterial sclerosis, and to changes in the liver, heart and brain following arterial disease. Lemoine and Lannois,[22] as already noted, have studied pancreatitis in four cases of diabetes and have thought that the new growth of interstitial tissue has its seat of origin in the perivascular tissue whence fibrous processes extend between the parenchymatous structures. An important feature of the inflammatory change described by them is the penetration of fibrous strands into the acini, separating the cells and producing what they designate unicellular sclerosis.

Hansemann[23] has attempted to define a variety of pancreatitis always associated with diabetes. The organ is diminished in size and is flattened from before back. Its interstitial tissue is in continuity with that of adjacent structures and consequently the removal of the organ is more difficult than usual. The microscope demonstrates an atrophy of the parenchymatous elements which are in part replaced by new fibrous tissue. He thinks that the lesion is similar to certain forms of granular atrophy of the kidneys.

Should there be, as Hansemann claims, a type of pancreatitis peculiar to diabetes, that is, a form of inflammation impairing the internal function of the gland, glycosuria would not ensue until the lesion had reached a certain grade of intensity, and in its earliest stage the lesion would not be accompanied by diabetes. On the other hand, when chronic interstitial pancreatitis, whatever the type may be, has destroyed a very large part of the parenchyma, one may expect diabetes; the specific type, should such exist, would be associated with the disease at an earlier stage.

For the purpose of the present study it is pertinent to inquire what histological changes are associated with the occurrence of diabetes. When a lesion of the pancreas is the cause of the disease, is the condition dependent upon changes in the acini or in the islands of Langerhans or in both? Total destruction of the acini is often accompanied by destruction or alteration of the interacinar structures, and rarely,

[22] Op. cit.

[23] *Zeitsch. f. klin. Med.*, 1894, xxvi, p. 191.

if ever, are the islands the seat of marked lesion while the acini are unchanged.

The islands of Langerhans are composed of columns of cells having no communication with the ducts of the gland, but in intimate relation with a rich capillary network. An analogous condition is found in the thyroid gland and in the adrenal. The pancreas, as do these organs, exerts through the medium of the blood an important influence on metabolism, and it has been suggested by several observers that the islands of Langerhans may furnish an internal secretion to the blood. Whether the gland furnishes some substance which aids carbohydrate assimilation or destroys some noxious product hindering it, is immaterial to the present study. Where diabetes is the result of pancreatic disease, do the islands exhibit lesions?

I have examined microscopically the pancreas from eleven cases of diabetes, and in four instances such marked change was found that one could not doubt the relationship of the general disease to the lesion of the organ. The limited number of cases makes far reaching conclusions impossible. Nevertheless, several facts of considerable interest appear.

Where (Case XVII) the pancreas was found to be the seat of advanced hyaline degeneration, the islands of Langerhans were universally involved in the process so that structures recognizable as interacinar islets were not discoverable. It is probable that the lesion had its origin in these bodies, though with its advance it has passed their limits. On the other hand, a considerable proportion of the secreting tissue, though the seat of fatty degeneration, was in a fair state of preservation and there was no hyaline deposit about its blood-vessels. Where the histological picture was not obscured by self-digestion, which is itself evidence of functional activity, the gland-cells were relatively normal in appearance. In this case fatal diabetes followed a lesion which had in great part obliterated the islands of Langerhans, though a considerable proportion of the intervening parenchyma was relatively intact.

Of eleven instances of chronic inflammation, classified as interlobular pancreatitis, in only one case (XIII) was the lesion accom-

panied by diabetes mellitus. Here glycosuria was of a mild type and disappeared when the individual was placed upon a diet from which carbohydrates were as far as possible eliminated. With this type of inflammation the islands of Langerhans are implicated only when the lesion has reached an advanced stage so that the organ may be markedly sclerotic while the interacinar structures are still unaltered.

Advanced parenchymatous degeneration and interstitial inflammation were found in three cases where obstruction of the ducts had been caused by calculi or by neoplasm (XI, XII and XIII). Diabetes of a mild type was present in one of these cases (XIII), and inflammatory atrophy of the gland was of an extreme grade. A very large proportion of the parenchyma had been replaced by fibrous tissue; the islands which persist are often embedded in dense sclerotic tissue containing no other epithelial elements and often show marked alterations. Compressed by the scar-like tissue, their cells are atrophied and thickened strands of stroma penetrate along their capillaries.

It is well known that obstruction of the pancreatic duct in human cases is accompanied by diabetes only when the consequent atrophy has caused great destruction of the gland. In animals it is extremely difficult, if indeed possible, to produce glycosuria by ligation of the ducts. These facts are explicable, should we assume that the islands of Langerhans, which resist the sclerosis, are the elements of the gland influencing carbohydrate metabolism.

In two of three cases, classified as interacinar pancreatitis, diabetes accompanied the lesion. Microscopic examination of the gland demonstrated less change than was observable in several instances of the interlobular type unattended by diabetes (Cases VII, XI and XII). Microscopic examination revealed the presence of advanced diffuse sclerosis. In accord with what one might expect from the diffuse interacinar character of the newly formed stroma, the islands of Langerhans are not spared but are surrounded and invaded by new tissue.

In one case of interacinar sclerosis (XVI) diabetes was not observed. The inflammation here accompanied the disease, hæmo-

chromatosis. This condition has been shown by P. Marie,[24] Anschütz[25] and Opie[26] to cause chronic interstitial inflammation of the pancreas, which, having reached a certain grade of intensity, is in turn followed by diabetes, the diabetes with pigmentation, or *diabète bronzé* of French writers. The pancreas in the case recorded weighed 170 grms. and the chronic inflammatory changes were not very advanced. The individual affected with hæmochromatosis died of intercurrent typhoid fever, and it is probable that his pancreatitis had not reached a sufficient intensity to cause diabetes.

SUMMARY AND CONCLUSIONS.

(1) Congenital syphilitic pancreatitis retards the development of the glandular acini but does not affect the islands of Langerhans. Embedded in the stroma, but not invaded by it, the latter maintain their continuity with the small ducts and acini with which they have a common origin.

(2) Two types of chronic interstitial inflammation affecting the developed pancreas are distinguishable:

(*a*) *Interlobular Pancreatitis.*—In the interlobular variety the inflammatory process is localized chiefly at the periphery of the lobule and implicates the islands of Langerhans only when the sclerotic process has reached a very advanced grade. When pancreatitis has followed obstruction of the ducts, the islands long remain unaltered though embedded in dense scar-like tissue.

(*b*) *Interacinar Pancreatitis.*—In the interacinar type the process is diffuse, invading the lobules and separating individual acini. The inflammatory change invades the islands of Langerhans.

(3) A relationship has been observed between lesions of the islands of Langerhans and the occurrence of diabetes mellitus.

(*a*) In one of eleven cases of interlobular pancreatitis diabetes of mild intensity occurred. The sclerosis, which in this case followed obstruction of the ducts by calculi, was far advanced and affected the islands of Langerhans.

[24] *Semaine méd.*, 1895, xv, p. 229.
[25] *Deutsches Arch. f. klin. Med.*, 1899, lxii, p. 411.
[26] *Journal of Experimental Medicine*, 1899, iv, p. 279.

(*b*) In two of three cases of interacinar pancreatitis, diabetes was present. The third case was associated with a condition, hæmochromatosis, which at a later stage is associated with diabetes, the result of pancreatic lesion.

(*c*) In a fourth case of diabetes, hyaline deposit between the capillaries and the parenchymatous cells had so completely altered the islands of Langerhans that they were no longer recognizable.

DESCRIPTION OF PLATES XXVII AND XXVIII.

PLATE XXVII.

Fig. 1.—Congenital syphilitic pancreatitis (Case I). Showing a cell-column of an island of Langerhans in continuity with a small duct.

Fig. 2.—Chronic interstitial pancreatitis following duct-obstruction (Case XI). Showing islands unchanged though embedded in sclerotic tissue.

PLATE XXVIII.

Fig. 3.—Chronic interstitial pancreatitis of interacinar type (Case XV). Showing the invasion of an island by the inflammatory process.

Fig. 4.—Hyaline degeneration of the pancreas (Case XVII).

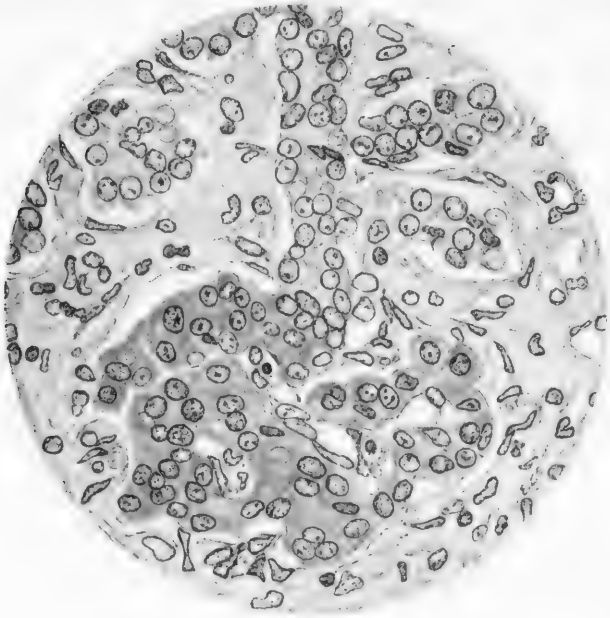

FIG. 1.

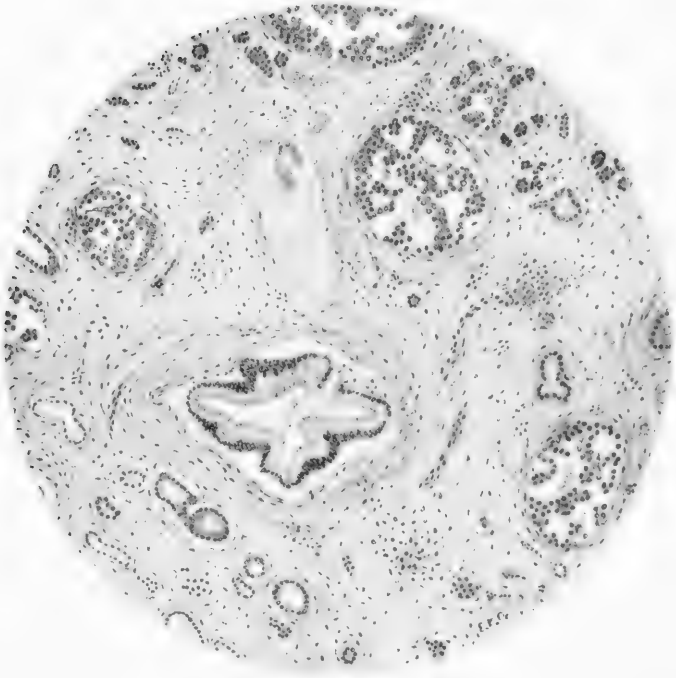

FIG. 2.

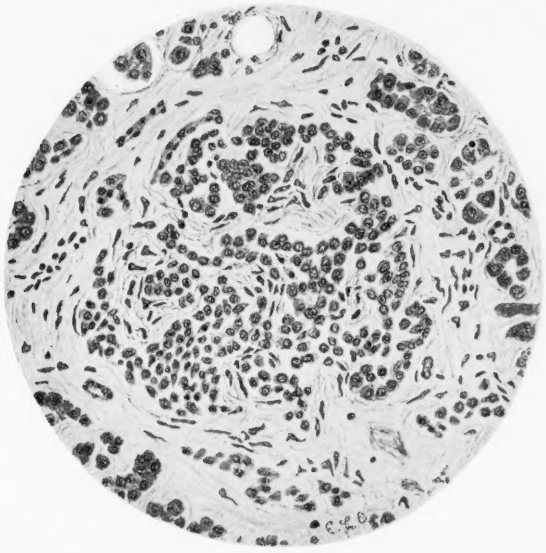

FIG. 3.

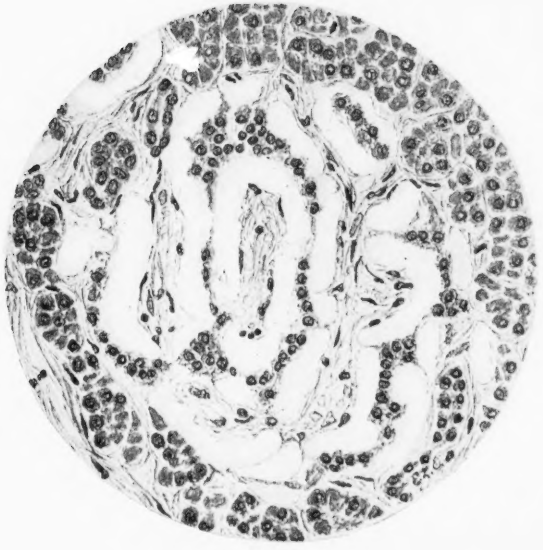

FIG. 4.